MONOGRAPHS ON BIOCHEMISTRY

EDITED BY

R. H. A. PLIMMER, D.Sc.

AND

F. G. HOPKINS, M.A., M.B., D.Sc., F.R.S.

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LONGMANS, GREEN AND CO.
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OXIDATIONS AND REDUCTIONS

IN

THE ANIMAL BODY

BY

H. D. DAKIN, D.Sc., F.I.C., F.R.S.

SECOND EDITION

LONGMANS, GREEN AND CO.
39 PATERNOSTER ROW, LONDON, E.C. 4
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1922

Dedicated

TO

CHRISTIAN A. HERTER

1865-1910

GENERAL PREFACE.

THE subject of Physiological Chemistry, or Biochemistry, is enlarging its borders to such an extent at the present time, that no single text-book upon the subject, without being cumbrous, can adequately deal with it as a whole, so as to give both a general and a detailed account of its present position. It is, moreover, difficult, in the case of the larger text-books, to keep abreast of so rapidly growing a science by means of new editions, and such volumes are therefore issued when much of their contents has become obsolete.

For this reason, an attempt is being made to place this branch of science in a more accessible position by issuing a series of monographs upon the various chapters of the subject, each independent of and yet dependent upon the others, so that from time to time, as new material and the demand therefor necessitate, a new edition of each monograph can be issued without re-issuing the whole series. In this way, both the expenses of publication and the expense to the purchaser will be diminished, and by a moderate outlay it will be possible to obtain a full account of any particular subject as nearly current as possible.

The editors of these monographs have kept two objects in view: firstly, that each author should be himself working at the subject with which he deals; and, secondly, that a *Bibliography*, as complete as possible, should be included, in order to avoid cross references, which are apt to be wrongly cited, and in order that each monograph may yield full and independent information of the work which has been done upon the subject.

It has been decided as a general scheme that the volumes first issued shall deal with the pure chemistry of physiological products and with certain general aspects of the subject. Subsequent monographs will be devoted to such questions as the chemistry of special tissues and particular aspects of metabolism. So the series, if continued, will proceed from physiological chemistry to what may be now more properly termed chemical physiology. This will depend upon the success which the first series achieves, and upon the divisions of the subject which may be of interest at the time.

R. H. A. P.
F. G. H.

PREFACE.

This small volume aims to give an account of the principal chemical reactions involving oxidation, or reduction, which are known to take place in the animal body. The subject is treated simply from the standpoint of the structure of the substances undergoing change.

The statements that fats and sugars are oxidized in the body to carbon dioxide and water, while proteins yield urea in addition, are no longer considered all-sufficient explanations of the chemical rôle of these substances in the animal economy. The study of chemical structure is rapidly changing the whole aspect of biological science, and we may confidently look forward to the time when the orderly succession of chemical reactions constituting the activities of the living cell will be resolved into their individual phases.

It is only within the last six or seven years that substantial progress has been made in unravelling, at least in part, the details of some of the simpler oxidation and reduction processes occurring in the animal body. But enough has been done to show that the problem is capable of successful attack by our present limited experimental methods. The significance of these investigations for the biological sciences, including medicine, hardly requires emphasis.

With the development of modern organic chemistry, the realization of the inadequacy of the common representation of chemical reactions by means of the usual formulæ becomes increasingly evident. The study of valency which is attracting investigators from all sides is likely to prove a most helpful aid in the adequate comprehension of reactions both *in vitro* and in the living organism.

H. D. D.

PREFACE TO SECOND EDITION.

Good progress has been made with the further unravelling of th complexities of biochemical oxidations during the eleven years tha have elapsed since the first edition of this monograph was published No particularly new methods have been evolved but greater anc more discriminating use has been made of existing methods parti cularly those relating to the study of changes in isolated organ such as the liver. The biochemistry of the carbohydrates has bee attacked with notable success and this section has been substantiall rewritten.

The inadequacy of the current methods of representing organi reactions with the ordinary formulæ becomes increasingly evident But until the newer conceptions as to the sequence of events in volved in chemical change are much further advanced, the biochemis will have to content himself with rigid representations which are con stantly felt to be only half truths. Perhaps when it is recalled tha the pure chemist is still debating as to what really happens when carbo monoxide burns in oxygen the biochemist may not feel so dissatisfie with the modest progress that has been made in elucidating bio chemical oxidations.

It has become increasingly clear in recent years that the oxi dations and reductions occurring in the living body are so closel interwoven with other types of reaction, especially condensation anc hydrolysis, that it would seem that their consideration apart from other metabolic changes was becoming unduly artificial and coulc not be justified much longer.

Scarborough-on-Hudson, New York. H. D. D.

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CHAPTER I.

I. INTRODUCTION.

THE evolution of heat as the result of vital activities and its cessation with death must have been obvious to man in the earliest stages of his development. It was recognized long ago that the phenomena of production of animal heat and the combustion of carbonaceous materials outside the body had much in common. The discovery of oxygen by Priestley and by Scheele (1771) first made it possible to form a rational conception of the chemical changes involved in combustion, but it was left for Lavoisier to clearly state the fundamental facts in a relatively modern form. The chemistry of the oxidations taking place in the animal body may fairly be said to date from the publication in 1780 of Lavoisier's "Experiences sur la respiration des animaux et sur les changements qui arrivent à l'air en passant par leur poumon". It was clearly shown by Lavoisier that oxygen was taken up by the lungs and in part converted into carbon dioxide, the carbon in Lavoisier's opinion being derived from the blood. The whole process was recognized as one of combustion and was believed to constitute the natural mechanism for the supply of animal heat. The necessity of furnishing the body with combustible material in the form of food in order to avoid injurious loss of body substance was clearly understood as was also the increased output of carbon dioxide following muscular activity.

Perhaps one of the most interesting results of Lavoisier's investigations is the fact that they led him to the conviction that vital processes are made up of a series of chemical reactions (1792). Unfortunately this idea was not revived until a much later date, and not until Liebig emphasized the importance for biology of the newly developing organic chemistry were material advances made. By degrees a clearer knowledge was obtained of the chemical nature of the fats, carbohydrates and proteins and of the laws governing their mutual interconversions in the body. One of the most important methods devised for the investigation of the metabolic activities of living organisms consists in the study of so-called "balances" in which

the intake and output of carbonaceous and nitrogenous substances are compared. Methods for the estimation of the final products of animal catabolism — carbon dioxide, water, urea, etc. — as well as of heat production have attained a remarkable degree of accuracy. But it is clear that such methods of study leave untouched the infinitely intricate problems of intermediary metabolism. It is relatively easy to obtain a balance sheet representing the intake and output of substances in the animal body, but what is fundamentally necessary for the proper appreciation of this balance sheet is a knowledge of the various chemical transactions which (to continue the simile) should be comprised in a trading account. For it is by the proper adjustment and regulation of these transactions that the energy represented by food and tissue substance are economically utilized according to the varying needs of the body. The rapidly developing appreciation of the fact that different proteins, fats and sugars are not physiologically equivalent but that certain definite chemical groups subserve special functions in the animal organism emphasizes the necessity of the study of intermediary metabolism.

A molecule of stearic acid taken into the body in the form of fat is known to undergo combustion so that eventually each of its eighteen carbon atoms will be converted into carbon dioxide. But no one imagines that such a change is immediate or direct—that every carbon atom simultaneously parts with its attached hydrogen atoms and by combining with oxygen yields carbon dioxide and water. The resolution of the fatty acid molecule is undoubtedly affected by a complicated series of chemical reactions following upon each other in definite sequence, and it is likely that most of these reactions are of a reversible character. A true knowledge of metabolic processes can only be obtained by the tedious unravelling of the complex system of biochemical changes into individual chemical reactions. At the present time only a few of these simple reactions have been recognized and studied, but even now it requires little imagination to realize that in the future it will be possible to construct an accurately itemized account of the animal body's chemical transactions both anabolic and catabolic. The value of such knowledge for the advancement of biology and medicine is sufficiently obvious.

Questions of chemical structure are paramount in these investigations, and the object of the following monograph is to give an account of some of the results which have been obtained by considering the oxidation and reduction processes of the animal body from the standpoint of the structure of the substances undergoing change. The

purely biological aspects and also the thermodynamics of the problems of oxidation and reduction have been necessarily omitted as outside the scope of the work. References to the many enzymes, catalase, oxygenases, peroxidases, etc., which, so far as is known, are without action upon the principal groups of substances which furnish energy to the organism have also been omitted.

Oxidation of saturated substances either in the laboratory or in the living organism usually consists in the replacement of hydrogen atoms by hydroxyl groups with formation of new substances which in many cases are capable of undergoing further decomposition. In the case of oxidations carried out in the animal body the primary products are so seldom capable of resisting further changes that it is commonly impossible to recognize the intermediate stages leading to the end-products of oxidation. The possibility of actually isolating intermediate products of oxidation from animal tissues and excretions is of course in large measure dependent upon whether the rate of their formation is great compared with the rate of decomposition. But in many cases in which it is impossible to detect intermediate products of oxidation under normal conditions, clues may be obtained as to their formation by indirect methods.

A consideration of the phenomena presented by the combustion of hydrocarbons is of interest in this connexion. Ordinarily a hydrocarbon burned with an adequate supply of oxygen yields nothing but carbon dioxide and water, without any indication of the formation of intermediate products of oxidation. But Bone and others have shown, by suitably modifying the conditions, that the oxidation of hydrocarbons by both slow and explosive combustion must be regarded as a process involving the initial formation of unstable hydroxylated molecules which subsequently undergo decomposition into simpler products. For example, formaldehyde and steam may be identified at an early stage in the combustion of methane, the formaldehyde decomposing at the high temperature into carbonic oxide and hydrogen, which are in turn oxidized to carbon dioxide and water. The changes are believed to take place as follows:—

$$CH_4 \rightarrow CH_3(OH) \rightarrow CH_2(OH)_2 \rightarrow CH_2O \rightarrow H_2 + CO \rightarrow H_2O + CO_2$$

The definite detection of these intermediate products of a reaction which usually results simply in the production of carbon dioxide and water is of great significance, for their formation is essentially analogous to what is believed to occur in the animal body.

The apparent absolute necessity for the participation of water at some stage in almost every oxidation is a fact of great significance.

As every one knows, the taking up by a substance of oxygen is called "oxidation," while parting with it is called „reduction;" and by convention, the loss of hydrogen by a substance is also termed oxidation, while gain in hydrogen is regarded as reduction. This convention tacitly implies the participation of water in some stage of the reaction, but it is only recently that much light has been thrown on the function of water in simple oxidations. It has long been known in terms of the electrolytic dissociation hypothesis that the oxidation of ions is synonymous with an increase in their positive ionic charge, or, what is equivalent, a reduction of the negative charge, while reduction involves the diminution of the positive or increase of the negative ionic charge. It was a rational assumption to suppose that the function of the water found to be necessary in many simple oxidations was concerned with the furtherance of ionic dissociation and that without ionic dissociation in the absence of water no chemical reaction would occur. It appears however that water plays a much more important rôle than that just indicated. With regard to chemical reactions generally including oxidations, it is becoming increasingly evident that the participating molecules are prone to unite with the formation of unstable additive compounds which then undergo intramolecular rearrangement and break down into the simple end products of the reaction.

Recently H. v. Wartenberg and Sieg (1920), utilizing earlier experiments of Wieland and others, have produeed the most positive and convincing evidence that the oxidation of the simplest carbon compound, carbon monoxide, to carbon dioxide by means of oxygen takes place in ihe following phases:

$$
\begin{aligned}
CO + H_2O &= H \cdot COOH \\
H \cdot COOH &= CO_2 + H_2 \\
H_2 + O_2 &= H_2O_2 \\
H_2O_2 &= H_2O + \tfrac{1}{2}O_2
\end{aligned}
$$

The above scheme effects a reconciliation between older views advanced by Dixon, Traube and others. Perhaps one of the most extraordinary observations concerned with this reaction is the fact that while water is absolutely necessary for the reaction, *free oxygen is not essential;* for Wieland showed that using palladium black in the absence of oxygen but in the presence of water, carbonic oxide could be oxidized to carbon dioxide at room temperature with liberation of hydrogen. It should be understood that the above scheme of reaction for the burning of carbonic oxide is not a figment of the chemist's imagination but is based on the actual isolation under

suitable experimental conditions of formic acid, hydrogen and hydrogen peroxide as intermediate products.

An analogous reaction undoubtedley takes place in the combustion of methane in oxygen, for Bone and Wheeler (1902) have detected the formation of large amounts of formaldehyde. It is probable that formaldehyde is not the first product but derived from an earlier more unstable addition compound and that it then undergoes simultaneous oxidation to carbonic oxide and carbon dioxide.

On glancing once more at the series of equations representing the combustion of carbon monoxide it will be noted that: *a*, A primary unstable addition compound (formic acid) is produced. *b*, Hydrogen is then separated from this addition compound. *c*, The hydrogen then unites or is "accepted" by free oxygen with the formation of a peroxide. *d*, The peroxide decomposes to give water with re-liberation of molecular oxygen in amount equal to half of that taking part in reaction *c*. It is reasonable to believe that if the simple reaction $CO + O = CO_2$ is in reality resolvable into four concurrent chemical changes, a closer analysis of biochemical oxidations will reveal similar analogies, and indeed they are already being discovered. It will be seen that in the reactions concerned with the oxidation of carbon monoxide to carbon dioxide, the most essential change consists in *the removal of hydrogen* from the formic acid resulting from the union of carbon monoxide and water and that oxidation, strictly speaking, only begins when the liberated hydrogen has to be dealt with. This conception of the lability of hydrogen as being at the root of many or, as some suppose, all oxidations is one of the newer ideas that is bound to affect the study of biochemical oxidations profoundly. These ideas have been largely but not exclusively developed by H. Wieland and according to ~~them~~ oxidation is to be regarded essentially as a process of dehydrogenation. Wieland's theory will be referred to in the next section.

Three independent studies on the oxidation of paraffins and especially paraffin wax have recently been published by Kelber, F. Fischer and Schneider and by Grün. The results appear of great significance from many points of view. It is found that paraffin wax which is so resistant to most oxidising reagents is readily oxidised at temperatures varying from $140^0 - 170^0$ by ordinary air or oxygen. Catalysts are apparently unnecessary and many of them have a negative action though manganese and iron appear to favour the change. The reaction is of course strongly exothermic and if it is not carefully controlled the carbon atoms are burned successively away so that a whole

series of fatty acids are formed from those of high molecular weight down to acetic and formic acids and finally carbon dioxide. Fischer and Schneider obtained principally normal fatty acids with an odd number of carbon atoms, $C_{13}H_{26}O_2$, $C_{15}H_{30}O_2$, $C_{17}H_{34}O_2$ and $C_{19}H_{38}O_2$, while Kelber obtained mostly acids with even numbers of carbon atoms. The successive burning away of pairs of carbon atoms illustrated by Fischer and Schneider's results is of extraordinary interest in connection with fatty acid catabolism in the body. It should be noted also that hydroxy acids and also acetone are products of the reaction just as they are found to occur in animal fatty acid metabolism. Kelber states that the same type of oxidation may be carried out starting with stearic acid instead of paraffin; and it is eminently desirable that experiments along these lines should be extended for they promise to throw much light on the analogous oxidation of fatty acids *in vivo*. In the following pages will be found a large number of cases of biochemical oxidations involving the similar formation of hydroxylated intermediate products. Thus, butyric acid may yield directly or indirectly β-hydroxybutyric acid and acetoacetic acid, the latter substance being formed from the hypothetical β-dihydroxybutyric acid by loss of water:—

$$\underset{\text{(Butyric acid)}}{CH_3\cdot CH_2\cdot CH_2\cdot COOH} \rightarrow \underset{(\beta\text{-Hydroxybutyric acid})}{CH_3\cdot CH(OH)CH_2\cdot COOH} \rightarrow \underset{(\beta\text{-Dihydroxybutyric acid})}{CH_3\cdot C(OH)_2\cdot CH_2\cdot COOH}$$

$$\downarrow$$

$$\underset{\text{(Acetoacetic acid)}}{CH_3\cdot CO\cdot CH_2\cdot COOH}$$

Similarly α-hydroxy and α-amino acids yield α-ketonic acids, while hypoxanthine (6-hydroxy-purine) gives on oxidation in the body successively xanthine (2·6-dihydroxy-purine) and uric acid (2·6·8-trihydroxy-purine).

It will be seen later that most of the striking biochemical oxidations of the living cell may be imitated more or less satisfactorily by experiments *in vitro*, and there is no evidence that suggests the oxidative processes of the living organism differ in any fundamental way from chemical oxidations known to take place in inanimate nature.

II. THE NATURE OF THE OXIDIZING AND REDUCING AGENTS OF THE ANIMAL BODY.

The substances undergoing active metabolism in the animal body, comprising the proteins, carbohydrates, fats, and their derivatives, are practically entirely resistant to oxidation by oxygen under ordinary conditions; yet in the animal body the carbon of all these types of compounds is readily oxidized to carbon dioxide. Until recently it was generally conceded that some process of activation of the atmospheric oxygen must take place in the body in order to account for the observed chemical changes. Oxygen loosely bound in the form of oxyhæmoglobin is an ineffective oxidizing agent, so that the main function of hæmoglobin appears to be that of a more or less indifferent oxygen transport agent.

Many theories have been propounded from time to time to account for the activation of oxygen, not only in biochemical oxidation but in other reactions as well. A detailed discussion of this question lies outside the scope of this monograph, but brief reference may be made to a few of the more important suggestions.[1]

As a result of his studies upon ozone Schönbein was inclined to seek the cause of the activation of oxygen in its polymerization, while many writers in more recent times have put forward the hypothesis that the activation of molecular oxygen is due to a separation of the oxygen molecule into atoms or ions (Clausius, Van't Hoff, and others). Hoppe-Seyler, and later Baumann, on the other hand, were inclined to ascribe the activation of oxygen in living cells to the resolution of the oxygen molecule by means of nascent hydrogen or other reducing agent, with formation of water and an atom of active

[1] For information upon these matters reference may be made to "Kritische Studien über die Vorgänge der Autoxydation," by C. Engler and J. Weissberg, Vieweg and Sohn, 1904, and to an excellent monograph by J. H. Kastle, "The Oxidases and other Oxygen-catalysts concerned in Biochemical Oxidations," Bulletin No. 59, Hygienic Laboratory, Public Health and Marine Hospital Service of the United States. Also G. Bodländer, "Ueber langsame Verbrennung," Ahrens' Vorträge, 1899, 385 (Enke, Stuttgart). The paper by Engler and Herzog (1909) contains an excellent historical summary.

oxygen. Hoppe-Seyler arrived at this hypothesis largely from a study of the changes involved in anærobic fermentation in which the formation of active hydrogen capable of effecting reduction is commonly observed. In support of his theory he showed how hydrogen absorbed by palladium in the presence of water and oxygen might effect many interesting oxidations as well as reductions. Examples of these oxidations are the oxidation of benzene to phenol and of toluene to benzoic acid—both of these reactions occurring in the animal body. There are, however, many difficulties in the application of Hoppe-Seyler's theory to the reactions occurring in animal cells.

The most generally accepted views upon auto-oxidation in general are based upon modifications of Moritz Traube's peroxide theory of oxidations. Traube introduced the idea of "oxygen carriers" — substances capable of uniting with molecular oxygen with formation of superoxides. In contrast with most other theories, no decomposition of molecular oxygen into the atomic or ionic condition is believed to occur. According to Traube the presence of water is an essential condition for the auto-oxidation of all substances. At first it was believed that hydrogen peroxide was the essential oxidizing agent in every case, but later, especially owing to the work of Engler and Wild and of Bach, it became generally recognized that a great variety of superoxides might be formed as intermediate stages in auto-oxidations. According to Engler and Weissberg, two main types of auto-oxidation may be recognized—the simplest of these is presented by the case of substances which at the same time induce the oxidative process (autoxidator) and are themselves oxidized. The second type of auto-oxidation is presented by reactions in which a third substance, not ordinarily capable of undergoing oxidation, is oxidized through the agency of a superoxide formed by the union of molecular oxygen with a reactive substance capable of undergoing auto-oxidation. A striking example of this type of change is presented by the oxidation of indigo-blue or similar substances by means of benzaldehyde and oxygen. Under ordinary circumstances indigo solutions are completely unaffected by oxygen, that is to say, no auto-oxidation occurs. When, however, benzaldehyde is present half of the oxygen taken up by the aldehyde while undergoing auto-oxidation with formation of benzoic acid is utilized in effecting the oxidation of indigo. The precise mechanism of the reaction has been experimentally determined by Baeyer and Villiger, and found to accord with the previously formulated theory of Bodländer. Benzaldehyde when undergoing auto-oxidation unites with a molecule of

oxygen to form benzoylhydrogen peroxide $C_6H_5 \cdot CO \cdot O \cdot OH$ (1), a substance possessing powerful oxidizing properties. In the absence of other oxidizable substances, this peroxide reacts with a second molecule of benzaldehyde forming two molecules of benzoic acid (2). In the presence of an oxidizable substance such as indigo, however, one atom of oxygen is appropriated for oxidizing the indigo, one molecule of benzoic acid being formed (3):—

(1) $C_6H_5 \cdot CHO + O_2 = C_6H_5 \cdot CO \cdot O \cdot OH$ (benzoylhydrogen peroxide).
(2) $C_6H_5 \cdot CO \cdot O \cdot OH + C_6H_5 \cdot CHO = 2\,C_6H_5 \cdot COOH$.
(3) $C_6H_5 \cdot CO \cdot O \cdot OH + \text{Indigo} = C_6H_5 \cdot COOH +$ oxidation products of indigo.

These reactions concerning the auto-oxidation of benzaldehyde are reproduced for the reason that they appear to present certain analogies with changes concerned with biochemical oxidations and reductions. It is generally believed that living cells contain labile substances capable of taking up molecular oxygen from the oxyhæmoglobin of the blood with the formation of unstable peroxides possessing marked oxidizing properties. Schönbein, and later Bach, have shown that a large number of substances of the most diverse kinds, when undergoing slow oxidation yield substances giving the reactions of hydrogen peroxide (cp. also Radziszewski). These peroxide-yielding substances include representatives of the following classes: elementary metals and non-metals such as hydrogen, phosphorus, zinc, etc., hydrocarbons, terpenes, alcohols, aldehydes, acids, carbohydrates, ethers, phenols, and aromatic bases and alkaloids. In addition, Baeyer and others have actually isolated a number of superoxides and substituted hydrogen peroxides derived from many different types of aldehydes and ketones. It certainly appears likely that substances of this type are concerned with the oxidations of substances in living tissues, and indeed such knowledge as has been derived from a study of the various oxidations effected by enzymes found in the living cells strongly supports such a supposition. The occurrence of certain metallic salts, especially those of iron and manganese, in conjunction with certain vegetable oxidases, and the extraordinary influence they have upon the ferment activity, is paralleled by the catalytic action of these same salts in accelerating oxidations *in vitro* by means of hydrogen peroxide (Bertrand, Fenton, Wolff, and others). According to Mummery the action of the metallic salts results in the formation of ionisable compounds with the peroxide. Röhrman and Shmamine have obtained compounds from mixtures containing proteins, hydrogen peroxide and ferrous salts which exhibit the usual reactions of peroxidases. They consider the possibility of similar compounds being

formed in the body and that their function is to raise the potential of oxygen associated with it.

Largely owing to the work of Bach and Chodat it has been commonly assumed that the oxidases concerned with biochemical oxidations represent systems composed of a superoxide formed by the union of ordinary oxygen with an "oxygenase" together with a catalyst (peroxidase) capable of acting upon it with production of active oxygen of high potential. The marked individuality of the animal as well as some of the vegetable oxidases makes it appear likely that both the peroxide and peroxidase must bear a special relation to the substance undergoing change. The specific character of animal oxidations is most remarkable, especially when phenomena such as those presented by diabetes and alcaptonuria are concerned. In these conditions oxidation of a single readily oxidizable product of metabolism (glucose, homogentisic acid) may be completely restrained without in the least impairing the capacity of the body for effecting the oxidation of other substances.

Within recent years other evidence has been secured in favour of the belief of the formation of unstable superoxides as the active oxidizing reagents of the body. If the hypothesis of superoxide formation is correct, one would expect a certain similarity between the oxidations effected in the body and those brought about by the simplest superoxide, namely hydrogen peroxide. As a matter of fact, an extraordinarily close similarity as regards the types of reactions exists between the two sets of phenomena. Thus the normal saturated fatty acids in the body undergo oxidation in the β-position, butyric acid yielding acetoacetic acid—a truly remarkable change.

Hydrogen peroxide alone of all the various chemical oxidizing agents brings about precisely the same reaction (p. 29):—

$$\underset{\text{(Butyric acid)}}{CH_3 \cdot CH_2 \cdot CH_2 \cdot COOH} \rightarrow \underset{\text{(Acetoacetic acid)}}{CH_3 \cdot CO \cdot CH_2 \cdot COOH}$$

Hydroxy acids, such as lactic acid and β-hydroxybutyric acid, are oxidized to ketonic acids, both in the body and by hydrogen peroxide (p. 72):—

$$\underset{\text{(β-Hydroxybutyric acid)}}{CH_3 \cdot CHOH \cdot CH_2 \cdot COOH} \rightarrow \underset{\text{(Acetoacetic acid)}}{CH_3 \cdot CO \cdot CH_2 \cdot COOH}$$

Amino acids and ketonic acids, such as leucine and phenylpyruvic acid, are oxidized to lower fatty acids with liberation of carbon dioxide and either ammonia or water (p. 65):—

$$\begin{matrix} CH_3 \\ CH_3 \end{matrix}\Big\rangle \underset{\text{(Leucine)}}{CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH} \rightarrow \begin{matrix} CH_3 \\ CH_3 \end{matrix}\Big\rangle \underset{\text{(Isovaleric acid)}}{CH_2 \cdot CH_2 \cdot COOH} + NH_3 + H_2O$$

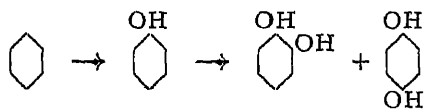
Glycerol may be oxidized by hydrogen peroxide to glyceric aldehyde or dihydroxyacetone. The same reaction is believed to occur in the body (p. 122):—

$$\underset{\text{(Glycerol)}}{CH_2(OH)\cdot CH(OH)\cdot CH_2(OH)} \rightarrow \underset{\text{(Glyceric aldehyde)}}{CH_2(OH)\cdot CH(OH)\cdot CHO}$$

Glucose may be oxidized in the body to glucuronic acid, while hydrogen peroxide is the only reagent capable of effecting this change outside the body (p. 116):—

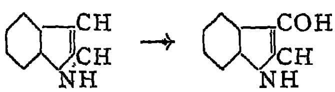
$$\underset{\text{(Glucose)}}{CH_2OH\cdot(CHOH)_4\cdot CHO} \rightarrow \underset{\text{(Glucuronic acid)}}{COOH\cdot(CHOH)_4\cdot CHO}$$

Benzene is oxidized in the body to phenol, catechol and quinol, and precisely the same change is brought about by hydrogen peroxide, but by scarcely any other reagent (p. 137):—



(Benzene) (Phenol) (Catechol) (Quinol)

Indole is oxidized to indoxyl in the body while no reagent other than hydrogen peroxide has been observed to bring about this change *in vitro* (p. 144):—



(Indole) (Indoxyl)

A great many other biochemical reactions of less strikingly characteristic types such as the oxidation of simple alcohols, aldehydes, acids, etc., may be reproduced *in vitro* by means of hydrogen peroxide.

An additional point of similarity between the types of oxidation effected by the animal tissues and by hydrogen peroxide is seen in the behaviour of the saturated and unsaturated acids. In the animal body the unsaturated acids, e.g. oleic acid, occur along with the corresponding saturated acids, and there does not appear to be a very profound difference in the ease with which the two types of acids undergo oxidation in the living organization. Indeed, in the case of some of the simpler acids the saturated acids appear to be more readily oxidized in the body. When these saturated and unsaturated acids are subjected to oxidation with most of the common laboratory reagents, the unsaturated acids are, of course, infinitely more readily oxidized than are the saturated acids, which appear remarkably stable. But if hydrogen peroxide be chosen as oxidizing agent, and is allowed to act on the

neutral salts of the saturated and unsaturated acids under comparable conditions, it is found that they both undergo oxidation to not widely different extents.[1]

The behaviour of some of the dibasic acids is also instructive. Oxalic acid, the simplest member of the group, although very readily oxidized by many laboratory reagents, is oxidized with great difficulty in the animal body. Malonic, succinic, and glutaric acids, on the other hand, are readily oxidized in the body although more stable than oxalic acid to many oxidizing reagents. On oxidizing the neutral salts of these acids with hydrogen peroxide, it is found that as in the body, oxalic acid is relatively little attacked, while its homologues are readily oxidized with liberation of carbon dioxide.[2]

It would seem, therefore, as if the evidence in favour of the hypothesis of superoxide formation in living cells is strong. It must not be inferred, however, that hydrogen peroxide is the active agent; indeed there is distinct evidence against such an assumption. It is very likely that hydrogen peroxide may be formed in small amounts during the processes of auto-oxidation occurring in the body, but the widely distributed enzyme catalase at once brings about its decomposition with liberation of molecular (inactive) oxygen. So far as is known, the other organic superoxides are unaffected by catalase.

Since the preceding account was written in 1912, a new conception of oxidation has been developed especially by Heinrich Wieland. According to this view oxidations and reductions are to be regarded essentially as reversible reactions in which hydrogen is either removed or added-dehydrogenation and hydrogenation. The experimental evidence in support of this idea is so strong and complete with regard to many types of oxidation that the theory requires serious consideration. The extension by Wieland of his theory to many types of biochemical reactions is not as convincing as might be, and has been vigorously contested by Bach, but the writer believes that taken in conjunction and not to the exclusion of the peroxide theories of oxidation it may help in the future to throw much light on the mechanism of biochemical reactions.

The reversibility of many catalytic reactions occurring at relatively high temperatures such as:

$$CH_2{=}CH_2 + H_2 \rightleftharpoons CH_3 \cdot CH_3$$
$$C_6H_6 + 3\,H_2 \rightleftharpoons C_6H_{12}$$

had long been known, but Wieland showed the same thing to be true for many reactions occurring at ordinary temperatures. For example,

[1] Unpublished observations. [2] Unpublished observations.

quinone is readily reduced by palladium and excess of hydrogen to hydroquinone. But on shaking oxygen-free palladium with hydroquinone solutions a partial reconversion into ~~hydro~~quinone was effected. The oxidation consists in the removal of labile hydrogen which is taken up by the palladium:

$$C_6H_4O_2 + H_2 \rightleftharpoons C_6H_4(OH)_2$$

Wieland furthermore produces suggestive evidence that the palladium not only serves as an acceptor for the hydrogen but forms a loose combination with the substance undergoing change very much in the same way as enzymes are believed to function.

It was next shown that the oxidation of aldehydes to acids, a reaction in which peroxide formation undoubtedly occurs under certain conditions as previously referred to, can also take place in an altogether different fashion without involving any activation of oxygen but rather the activation of hydrogen and its removal by catalysts. Thus, on shaking *moist* acetaldehyde in the absence of air with palladium black, acetic acid is obtained while the hydrogen is bound by the palladium. The presence of moisture was found to be essential not only for the reaction under discussion but also for the oxidation of acetaldehyde and similar substances by silver oxide — a reagent in general use for the oxidation of aldehydes. The conclusion was drawn that the acetaldehyde reacts in its hydrate form and is then dehydrogenated by the palladium. The fact that chloral hydrate but not chloral itself reacted similarly supports this view. The oxidation of acetaldehyde by palladium in the absence of air may therefore be represented as follows:

$$CH_3 \cdot COH \rightarrow CH_3 \cdot CH\langle{}^{OH}_{OH} \rightarrow CH_3 \cdot COOH + H_2.$$

The reaction naturally comes to a standstill at an early stage unless means are taken to remove the hydrogen taken up by the palladium. This may be done either by the admission of oxygen when the hydrogen is burnt to water or, what is more important, by the addition of substances such as benzoquinone, or methylene blue which act as acceptors of hydrogen. Under these conditions the oxidation can rapidly proceed to completion. The possibility of reactions of this type being concerned in biochemical changes was obvious and Wieland then turned to a consideration of reactions with substances of physiological importance. He found that glucose could be oxidised to carbon dioxide and water at low temperatures using either oxygen or quinones as acceptors for hydrogen. In the latter case the combustion could be effected in the complete absence of elementary oxygen. Lactic acid was similarly oxidised to pyruvic acid,

and various other oxidations of phenols, aromatic amines, characteristic of so-called 'oxidase' reactions were successfully imitated. Finally the oxidation of alcohol to acetic acid in the absence of air but in the presence of methylene blue or quinone, was effected by substituting acetic acid bacteria for the palladium. On the other hand tyrosine and uric acid were not susceptible to dehydrogenation.

The newer investigations into the mechanism of alcoholic fermentation by which it has been farily definitely settled that pyruvic acid is the precussor of alcohol and that the production of pyruvic acid is dependent on the presence of a hydrogen acceptor normally provided by the acetaldehyde resulting from its decomposition, show clear analogies with Wieland's ideas:

$$C_6H_{12}O_6 \rightarrow 2CH_3 \cdot CO \cdot COOH + 2H_2$$
$$CH_3 \cdot CO \cdot COOH \rightarrow CH_3 \cdot CHO + CO_2$$
$$CH_3 \cdot CHO + H_2 \rightarrow CH_3 \cdot CH_2OH.$$

Objection has been taken by Bach to the views of Wieland as regards biochemical reactions and the former holds to his views that oxidation takes place by direct union with oxygen and also by the removal of water with addition of hydroxyl groups with simultaneous functioning of hydrogen acceptors. He regards many typical reactions as incapable of explanation on the basis of Wieland's theory of dehydrogenation. A final verdict as to the merits of the rival theories is not at present possible and it would seem not improbable that both will be found necessary.

With regard to the mechanism of biochemical reductions but little can be said. It appears that in some cases at any rate, the reduction of one molecule of substance takes place with simultaneous oxidation of a second molecule, so that the net result of the whole may be an exothermic hydrolysis. The Cannizzaro reaction (p. 141) which takes place in the body is an example of such a change. Two molecules of an aldehyde undergo rearrangement with formation of one molecule of an alcohol (reduction) and one molecule of an acid (oxidation):—

$$2R \cdot CHO + H_2O = R \cdot CH_2OH + R \cdot COOH$$

The types of reduction which are known to occur in the body are mostly of a kind which may be readily reproduced *in vitro*. The reduction of α- and β-ketonic acids and of ketones to the corresponding hydroxy-compounds and the reduction of the ammonium salts of α-ketonic acids to α-amino acids are well-established biochemical reactions. But the mechanism of these reductions by the organism is not understood. It is possible that two molecules of the ketonic compound

undergo simultaneous reduction and oxidation, or it may be that the reduction of the ketonic compound is brought about by the oxidation of some other labile easily oxidized substance. The striking investigations of Ciamician and Silber have shown that the reduction of many types of compounds when dissolved in alcohol and exposed to sunlight is effected with the simultaneous oxidation of a portion of the alcohol to acetaldehyde. In addition, Bach has recently shown how acetaldehyde may assist in the catalytic reduction of nitrates to nitrites by certain enzymes. Schardinger's observations on the reduction of methylene-blue in the presence of aldehydes may also be recalled. The interpretation of this reaction in terms of Wieland's hypothesis makes the reduction of the methylene blue secondary to the catalytic oxidation of the aldehyde by dehydrogenation. At present there appears to be little ground for believing in special reducing enzymes in the animal body and such reductions as are observed appear to be due to coupled reactions in which the necessary energy is supplied by a simultaneous oxidation.

Within the last few months announcement has been made of the isolation and identification of an autoxidizable substance which bids fair to be of altogether exceptional importance in relation to the oxidations and reductions occurring in living cells. The compound has been shown by Hopkins (1921) to occur in most if not all actively living organisms, both animal and vegetable, and hence may lay claim to general significance. The substance which is thermostable and not hydrolyzed by proteoclastic ferments is made up by the union of glutamic acid and cystine (or cysteine). The combination of the two amino-acids occurs through the union of an amino group of one acid with a carboxyl group of the other with removal of water, but three possibilities exit for glutamic acid-cystine combination of this type and the exact allocation of the position of union awaits final determination and confirmation by synthesis. The reduced form of the substance, corresponding to cysteine, and the oxidized form, corresponding to cystine, may be represented as follows, *G* representing a glutamic acid nucleus attached either to the amino or carboxyl groups of the cystine:

$$\begin{array}{lcl}
CH_2SH & \qquad & CH_2-S-S-CH_2 \\
| & & |\qquad\qquad\quad | \\
CHNH\ldots. & & CHNH\ldots.\quad CHNH\ldots. \\
| & & |\qquad\qquad\quad | \\
CO\ldots G & & CO\ldots.G\qquad CO\ldots.G
\end{array}$$

It will be seen that the passage from the reduced to the oxidized form involves the loss of a hydrogen atom and a doubling of the

molecular weight, and vice versa. The change of the reduced to the oxidized form takes place under the influence of ordinary oxygen apparently with intermediate function of hydrogen peroxide which in turn gives water and inactive oxygen. On the other hand fresh tissues can reduce the oxidized di-sulphide form to the simple sulphydric compound.

$$\mathrm{R \cdot CH_2 \cdot SH} \quad \mathrm{HS - CH_2 \cdot R} + \mathrm{O_2}$$
$$\downarrow$$
$$\mathrm{R \cdot CH_2 \cdot S - S \cdot CH_2 \cdot R + H_2O_2}$$
$$+ \mathrm{H_2}$$
$$\downarrow$$
$$\mathrm{R \cdot CH_2 \cdot SH + SH \cdot CH_2 \; R}$$

This pair of substances, readily interconvertible under conditions prevailing in the cell, possess precisely the properties which a co-ferment adapted to an oxidase system would be expected to possess and as such they occupy thus far a unique position. The reduced (SH) form present in almost all living cells can take up molecular oxygen while the oxidized (S-S) form so produced can act as a hydrogen acceptor and so catalyze oxidation reactions of the type described by Wieland to which reference has already been made.

The following experiments of Hopkins afford convincing evidence that glutathione plays a real part in cell dynamics. Fresh tissues of course reduce methylene blue and so does the reduced glutathione. On the other hand fresh tissues reduce the oxidized glutathione and from this it would at first appear that the tissues had a greater reduction (lower oxidation) potential than that due to the SH group of glutathione. Hopkins has found, however, that as a matter of fact the relations depend upon the hydrogen ion concentration of the medium. If the oxidized glutathione and fresh tissue are added to a methylene blue solution which is even very slightly on the acid side of neutrality, e. g., pH — 6.8 —the reduction of the dye is greatly slowed. The glutathione under these conditions is simply acting as a hydrogen acceptor, competing with the methylene blue in this respect and delaying or preventing the reduction of the latter. If the reaction of the medium is changed to pH — 7,4 or slightly greater, the normal rate of reduction of methylene blue by tissues in the presence of oxidized glutathione is greatly accelerated. The results are explained on the assumption that in the acid medium the S-S group of the glutathione acts simply as a hydrogen acceptor and the reduced compound thus formed is too stable to transfer its hydrogen to another acceptor. In neutral or alkaline solution this

transference does take place and the hydrogen is used in the reduction of methylene blue. The important fact follows from these observations that the two reactions concerned in the transference of hydrogen to the S-S group oxidized in glutathione under the influence of a tissue enzyme and its subsequent transference to the methylene blue, have a greater velocity than the direct reduction of methylene blue by the tissues. The function of the glutathione is thus essentially catalytic and the substance must be regarded as a co-enzyme.

The concentration of glutathione in the tissues is very low but as already stated its distribution is extremely wide. The blood proteins appear to contain vanishingly small amounts or possibly none and this observation agrees with the growing conviction that scarcely any oxidation of moment occurs in this medium. Yeast furnishes useful material for the preparation of glutathione though animal organs may serve equally well. The process of separation is laborious and difficult and depends largely on the skilful use of metallic salt precipitants including mercuric sulphate, which Hopkins has previously turned to good account. Fortunately a qualitative test already described by Mörner and by Heffter for sulphydryl compounds has been found applicable which does not require previous separation of the glutathione. This reaction consits in the production of a purple or permanganate color when the tissue or substance to be tested is suspended in saturated ammonium sulphate solution and sodium nitroprusside followed by excess of ammonia is added. This color reaction is only given by the reduced glutathione and not by the disulphide form. With the use of this reaction it possible to show the absence of glutathione in the fresh hen's egg but a thirty-six hour chicken embryo gives a strong reaction while the surrounding material gives none. Preliminary experiments appear to show that tumor cells contain less glutathione than neighboring tissues. The further investigation of glutathione will be awaited with the greatest interest since there is good reason to believe that its study may reveal many additional important facts in connection with the respiratory changes in living tissues.

Certain observations made by Meyerhof (1918) on oxidation processes in dead yeast cells and yeast extracts acquire new interest in the light of Hopkins' investigations. Meyerhof found that respiratory changes practically ceased when acetone-yeast preparations were exhaustively washed with water, but that they could be revived by the simple addition of the aqueous extract to the killed yeast. He furthermore showed that these washings gave qualitative reactions

indicating the presence of a compound containing the SH group but did not succeed in further defining the substance. Meyerhof was also able to show that washed killed yeast in which respiratory changes were in abeyance could be stimulated to carry on oxidation reactions by the addition of thioglycollic or α-thiolactic acid, although a careful analysis of the change induced by these thio-acids showed that it was not identical in intensity with that evoked by the yeast washings containing sulphydryl compounds and moreover differed in some other respects. It seems possible that the activator to respiratory activity in killed washed yeast, present in the aqueous washings observed by Meyerhof, is related to Hopkins glutathione.

Glutathione has undoubtedly connections with the "philothion" of de Rey-Pailhade described many years ago. It will be recalled that this author found that certain proteins and many tissues had the property of liberating hydrogen sulphide when digested with finely divided sulphur. The reaction was originally regarded as a fermentative reductase reaction. It is interesting to note that Heffter and Hausmann (1904) expressed the view that the reaction had great similarity to the production of hydrogen sulphide from mercaptans or other sulphydryl compounds:

$$2R \cdot SH + S = H_2S + R \cdot S - S \cdot R$$

That glutathione is the sulphydryl compound present in tissues and capable of bringing about the above reaction has been definitely proved. Heffter (1907) showed that an aqueous extract of acetone yeast contained an unidentifed compound containing a (SH) group as judged by positive nitroprusside reactions and that the extract could exert the reducing properties ascribed to "philothion". The autoxidizable properties of sulphydryl compounds and their possible function in the cell as oxygen acceptors and as catalytic reducing agents was also emphasized by Heffter who regarded them as "pseudoöxidases" according to Engler's scheme of classification.

Thunberg (1920) made some highly suggestive studies on the oxidation or rather dehydrogenation of intermediary products of metabolism. He found that when the finely chopped muscle of a freshly killed frog is thoroughly washed with water it loses its power to reduce methylene blue in the absence of oxygen. When, however, certain metabolites which do not themselves reduce the dye are added to the washed muscle the power to decolorize the methylene blue is restored wholly or in part. The change is represented by Thunberg as due to the action of specific enzymes which have the power to

effect the removal of hydrogen from the metabolite and transfer it to the methylene blue which is then reduced. The ferments are given the generic name of "hydrogentransportases". Under normal conditions the hydrogen is supposed to be burned to water or used for the reduction of other compounds. The specificity of the enzymes is rather unconvincingly inferred from their unequal resistance to thermal changes. Thunberg regards all food substances as essentially „hydrogen donators" and hydrogen is regarded as the common fuel of tissue combustions. Dehydrogenation, the addition and removal of water and the splitting off of carbon dioxide are regarded as the fundamental catabolic changes undergone by substances containing carbon, oxygen and hydrogen. The fact that many metabolites react with washed muscle tissue in the way described is certainly an interesting phenomenon; but whether, as Thunberg appears to suggest, all substances so reacting are thereby of necessity to be regarded as intermediary metabolites with specific „hydrogentransportases" to act upon them, appears much more doubtfoul. Indeed an inspection of Thunberg's results with analogously constituted substances only serves to increase the reviewer's hesitation in accepting the method as a means of deciding whether a substance is an intermediary metabolite or not. But whatever the outcome may be it is clear that Thunberg has hit upon an interesting mode of experimentation which should lead to further results of value.

Catalase. A word must be said with regard to catalase. The distribution and mode of action of this ferment are such that so far as the writer can see there is not a trace of evidence available suggesting that catalase is directly concerned with oxidation. It will be recalled that catalase only liberates inactive molecular oxygen when decomposing hydrogen peroxide and so far it has not been shown to accelerate or participate in the oxidation of any known metabolite. Within recent years it has been shown repeatedly that great increase in oxidation such for example as that which follows the fertilization of the sea urchins's eggs, is not accompanied by any comparable increase in catalase action (Amberg and Winternitz). Similar results have been obtained by Stehle and McCarty (1920) and by Seymour (1920) and others. Yet in spite of the failure to demonstrate the causal relation of catalase to any known oxidation a number of papers have been published within the last few years in which the implication is made that quantitative relations exist between the amount of catalase and the capacity of tissues to effect oxidations. Little regard is paid to factors such as variations in hydrogen ion concentration or changes

in the proportion of erythrocytes in the tissues examined by catalase, in spite of the fact that it appears that quantitative estimations of catalase are by no means easily carried out. Yet we are asked to believe that "catalase is the enzyme in the body principally responsible for oxidation" and that the fundamental problems of tissue oxidation can be solved by noting variations in its concentration. In the judgment of the writer it appears reasonable to reject the inferences drawn from these studies until unequivocal evidence is produced that catalase actually participates in the oxidation of known metabolic products and until the quantitative estimations of the catalase are made under more exactly controlled conditions.

It will be recalled in connection with Wielands's theory of oxidation by dehydrogenation, that activated hydrogen is removed by catalytic action from the substance undergoing oxidation. It should be noted that when oxygen acts as an acceptor for hydrogen, hydrogen peroxide and not water is the initial product even though the peroxide is at once further decomposed as in the explosion of hydrogen and oxygen gases:

$$H_2 + O_2 = H_2O_2$$
$$2\,H_2O_2 = 2\,H_2O + O_2$$

It is therefore reasonable to suppose that hydrogen peroxide may be transitorily formed in tissue oxidations when molecular oxygen acts as acceptor for activated hydrogen. The old idea that catalase may serve to prevent excessive accumulation in the tissues of injurious peroxide by converting it into water and inactive molecular oxygen seems to have a good deal to recommend it.

III. METHODS OF INVESTIGATION.

The methods employed for characterizing the oxidations and reductions which various substances undergo in the animal body are relatively few and simple. In the case of oxidations the first step in the investigation consists in determining the *end-product* of the reaction. This is usually a simple problem and may generally be determined by administering small quantities of the substance under investigation to an animal, preferably by subcutaneous injection.[1] The urine excreted during the period following the administration is specially examined for possible derivatives of the substance and also for the unchanged compound. Negative results from these experiments are usually indicative of the complete oxidation of the substance. In such cases it may be necessary to make special metabolism experiments in which the carbon and nitrogen balances are determined. When an organic substance has undergone complete oxidation in the animal organism, the carbon and nitrogen are approximately quantitatively converted into carbon dioxide and urea respectively.

The second step in the investigation aims at the determination of the intermediate steps in the process by which the substance has been converted into its end-products of oxidation. This is a very much more difficult problem than the first. By utilizing a number of methods it is usually possible definitely to determine the occurrence of certain reactions, to recognize the possible or probable occurrence of others and finally definitely to exclude a number of other reactions which theoretically might be concerned in the sequence of changes.

The first method commonly made use of may be termed a "method of exclusion" and depends upon the testing of provisional hypotheses constructed by analogy with chemical experiments *in vitro*.

[1] Subcutaneous injection is usually preferable to administration by mouth owing to the avoidance of possible bacterial decomposition in the alimentary tract prior to absorption. The method may present difficulties in the case of sparingly soluble substances. The use of dilute alcohol or sterile olive oil as solvents, or the conversion of the substances into soluble derivatives, salts, etc., may obviate the difficulty. Intraperitoneal injection is occasionally useful, but in some cases absorption is slow.

An example will render this clear. Acetic acid is oxidized in the body with great ease, yielding as end-products carbon dioxide and water. Now it is known that in the laboratory acetic acid may be oxidized to oxalic acid by a variety of oxidizing reagents (e.g. alkaline permanganate) and that by other reagents (e.g. permanganic acid) the oxalic acid thus formed may be oxidized to carbon dioxide and water. The question arises: Is oxalic acid an intermediate product in the oxidation of acetic acid in the animal body? The answer appears to be in the negative since on administering oxalic acid to an animal under conditions similar to those prevailing in the experiments with acetic acid, it is found that not only is the oxalic acid much more toxic than acetic acid but undergoes oxidation in the body with great difficulty compared with acetic acid. Oxalic acid is therefore probably *not* an intermediate product of the oxidation of acetic acid in the animal organism. On the other hand, acetic acid may be oxidized to formic acid by purely chemical means (hydrogen peroxide). Can formic acid be regarded as a possible stage in the biochemical oxidation of acetic acid? On testing the behaviour of formic acid in the animal body, it is found to undergo oxidation yielding the same end-product as acetic acid, namely carbon dioxide and water, and also to resemble acetic acid reasonably closely as regards toxicity and ease of oxidation. The inference to be drawn therefore is that formic acid *may be* an intermediate product of the oxidation of acetic acid in the animal organism.

It is clear that this "method of exclusion" is beset with great limitations since it is perfectly possible for substances of high toxicity to be constantly produced and rapidly transformed into other bodies so that the relative concentration of the toxic substance is always low. The production of formaldehyde in the photosynthetical processes occurring in green leaves is a case in point. Moreover, the behaviour of a substance when gradually produced, at low concentration and rapidly undergoing further change, may be very different from that of the same substance when rapidly injected in relatively high concentration into the tissues of an animal. Indeed, in the latter case it is always uncertain whether the substance ever really reaches the sphere of action in the cells normally concerned with its metabolism.

The second method is intimately bound up with the first and essentially based upon experience gained by a study of the behaviour of substances of biological importance under a variety of conditions. An attempt is then made to apply the knowledge thus gained to the elucidation of biochemical transformations. An example will illustrate

the method. It had long been known that administration of butyric acid to a diabetic animal was followed by the excretion of acetoacetic acid. For a long time the reaction was regarded as an indirect one—it was believed that the butyric acid in some way was broken down into smaller molecules which under suitable conditions might undergo synthesis with formation of acetoacetic acid. No chemical analogy was known for the conversion of butyric acid into acetoacetic acid by direct oxidation. It was subsequently found, however, that by a suitable choice of reagent, namely hydrogen peroxide, this type of reaction could be readily brought about *in vitro* and it is now generally conceded that the same reaction takes place in the animal body.

Many additional examples of the results of this type of investigation will be found in succeeding chapters.

A third method of obtaining information about intermediate products of biochemical oxidation is based upon the following consideration. For any particular substance under investigation it is possible to determine by trial the amount which on administration to an animal undergoes practically complete oxidation to its end-products. If in a second experiment a quantity of substance considerably larger than that which can be completely oxidized is administered to the animal, it will be found that in some cases the urine, in addition to unchanged substance, will contain compounds which appear to be intermediate products of oxidation. The possibility of isolating intermediate products by this direct method obviously depends upon the relative rates of oxidation of the parent substance and the intermediate products under the particular conditions of the experiment. The necessity of employing large quantities of substance make the conditions of experiment somewhat abnormal and the results must be accepted with some caution.

As examples of the method, reference may be made to the formation of intermediate products from β-phenylpropionic acid (p. 30) and the isolation of uric acid as an intermediate product in the oxidation of xanthine to allantoine (p. 130).

A fourth method of investigation is based upon the study of the action upon oxidizable substances of isolated surviving organs, or of the crushed tissue pulp or cell juices or aqueous extracts of various organs. The investigation of the oxidations which may be effected by the surviving liver when perfused with oxygenated blood has proved specially valuable. Embden's work on acetoacetic acid formation from fatty acids (p. 30) and Wiechowski's demonstration of the oxidation of uric acid to allantoine (p. 128) are excellent examples of this

method. By the use of isolated organs or organ extracts the tendency of the intact animal to effect complete oxidation of the substance is often avoided and the chance of detecting intermediate products is consequently increased. In recent years the method of liver perfusion has been of the greatest service in elucidating some of the details of carbohydrate metabolism.

A constant difficulty in determining the formation of intermediate products is the ease with which these substances undergo further change and so avoid detection. Knoop conceived the idea of introducing into the substance under investigation a resistant radical which would therefore be excreted in combination with some part of the molecule of the original substance. He studied the fate in the animal organism of fatty acids in which a phenyl group had been introduced in the position furthest removed from the carboxyl group, e.g. phenylacetic acid, β-phenylpropionic acid, γ-phenylbutyric acid and δ-phenylvaleric acid. Knoop found that the aliphatic side-chain underwent oxidation in the body in such a way that either benzoic or phenylacetic acid was excreted in combination with glycine. These important results will be referred to later.

The study of the fate of various substances in the animal organism under pathological conditions, especially human or experimental diabetes, has proved very fruitful. Experiments of this kind have brought to light many interesting relationships. Thus it has been found that many amino acids derived from proteins are converted into glucose in the diabetic organism while others yield the so-called "acetone bodies" β-hydroxybutyric acid, acetoacetic acid, and acetone (p. 75).

Investigation of the fate of various amino acids in cases of cystinuria, alcaptonuria (p. 84), and melanuria (p. 97), conditions which are associated with peculiar metabolic abnormalities, has also given valuable results. It is always difficult, however, to decide how closely reactions observed under pathological or abnormal conditions resemble those occurring in the normal organism.

The administration to animals of certain foreign substances often results in the excretion of these substances in combination with a second substance derived from the tissues of the organism. The reaction is frequently of the nature of a protective mechanism, the compound excreted being commonly less toxic than the original drug. In some cases the second substance derived from the tissues appears to be an intermediate product of normal metabolism. Examples of the use of this pharmacological method are found in the excretion of glucuronic acid derivatives when many aromatic alcohols and

ketones are administered to animals (p. 116). The glucuronic acid is undoubtedly derived from glucose and is an intermediate product of oxidation. A similar example is furnished by the behaviour of bromobenzene when administered to dogs. The substance is excreted in the form of bromophenylmercapturic acid, the sulphur-containing group being derived from the protein derivative, cysteine. The limitations of this method are of course great and the correlation of the results with the processes of oxidation in the normal organism in the absence of foreign substances is always difficult.

CHAPTER II.

I. THE OXIDATION OF FATTY ACIDS.

THE fats undergoing catabolism in the animal body are mainly derivatives of straight chain normal fatty acids containing an even number of carbon atoms. The number of carbon atoms varies from four (butyric acid) to twenty-four (carnaubic acid) or more. Palmitic, stearic, and oleic acids with sixteen and eighteen carbon atoms are quantitatively predominant. Formic acid and acetic acid and possibly propionic acid also occur in the body but not in the form of fats. All of these fatty acids undergo complete oxidation in the animal body with formation of carbon dioxide and water (Wöhler, Buchheim, Schotten, Pohl, Bergell and others). Schotten gave from 10 to 20 grms. of the fatty acids from formic to caproic acid to dogs by mouth in the form of sodium salts. The caproic, valeric, and butyric acids were practically completely oxidized, while about 10 per cent. of the acetate and 25 per cent. of the formate was excreted unchanged. Large quantities of sodium carbonate were present in all the urines. It has since been shown that when smaller amounts of acetates and formates are administered they are more completely oxidized in the body[1] (Pohl, Mallèvre, Pellacani). With the exception of formic acid, all of these saturated fatty acids are very resistant to the action of the ordinary chemical reagents, yet in the animal body they undergo oxidation with extraordinary ease. It appears that the liver is the main seat of at any rate the initial reactions involved in the oxidation of the higher fatty acids. What is the mechanism by which these fatty acids are oxidized? The complete oxidation of the molecule of a higher fatty acid to carbon dioxide and water must necessarily involve the successive formation of a series of intermediate substances. The detection and identification of these substances is an extremely difficult problem owing to the ease with which they undergo further change.

It is only since 1904, the year when Knoop published his

[1] It is often stated that formates and acetates are less readily oxidized than the higher acids. In the absence of information as to the relative rate of excretion of these salts by the kidneys, this conclusion is unwarranted.

important paper upon "Der Abbau aromatischer Fettsäuren im Tierkörper", that any material success has been obtained in the experimental investigation of fatty acid catabolism.

Knoop's Theory of β-Oxidation.—The ease with which the intermediary products of fatty acid catabolism undergo complete oxidation and so escape detection led Knoop to study the fate of fatty acids in which a radical had been introduced which was resistant to oxidation in the body. He chose among other substances the phenyl derivatives of acetic, propionic, butyric, and valeric acids. Phenylacetic acid does not undergo oxidation in the animal body but combines with glycine to yield phenaceturic acid[1], $C_6H_5 \cdot CH_2 \cdot CO \cdot NH \cdot CH_2 \cdot COOH$. β-Phenylpropionic acid, on the other hand, is oxidized to benzoic acid, which unites with glycine to form hippuric acid (E. & H. Salkowski). Knoop concluded that the oxidation of the side-chain of three carbon atoms in phenylpropionic acid did not take place with intermediate formation of a two-carbon side-chain (phenylacetic acid) as in this case the excretion of phenaceturic acid would have followed. Two carbon atoms appeared to have been removed from the phenylpropionic acid side-chain at one time.

γ-Phenylbutyric acid, on the other hand, was converted into phenaceturic acid, two carbon atoms being again removed from the fatty acid side-chain. δ-Phenylvaleric acid gave hippuric acid with loss of four carbon atoms and Dakin subsequently proved that this took place in two stages in each of which two carbon atoms were removed. The results may be represented as follows:—

Acid Fed.	Oxidation Product,	Excreted as
Benzoic acid, $C_6H_5 \cdot COOH$	Not oxidized	Hippuric acid
Phenylacetic acid, $C_6H_5 \cdot CH_2 \cdot COOH$	Not oxidized	Phenaceturic acid
Phenylpropionic acid, $C_6H_5 \cdot CH_2 \cdot CH_2 \cdot COOH$	$C_6H_5 \cdot COOH$	Hippuric acid
Phenylbutyric acid, $C_6H_5 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH$	$C_6H_5 \cdot CH_2 \cdot COOH$	Phenaceturic acid
Phenylvaleric acid, $C_6H_5 \cdot CH_2 \cdot CH_2 \cdot CH \cdot CH_2 \cdot COOH$	$C_6H_5 \cdot COOH$	Hippuric accid

On the basis of these experiments Knoop founded his theory of β-oxidation. According to this theory oxidation of the side-chains of these normal phenyl-fatty acids takes place in such a manner that the hydrogen attached to the β-carbon atom is selected for oxidation.

[1] The failure of phenylacetic acid to undergo oxidation in the body was ascribed by Baumann to the protection afforded to the CH_2 group by two non-oxidizable groups, C_6H_5 and COOH. It is more likely, however, that its ready condensation with glycine to form phenaceturic acid is in part responsible, since the glycine derivatives of phenyl-fatty acids are very stable substanzes. Thierfelder and Sherwin have shown that in man phenylacetic acid is excreted chiefly as phenacetylglutamine.

In this way the side-chains are reduced by the loss of two or some multiple of two carbon atoms at each successive step:—

$$C_6H_5{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2 \,\vdots\, CH_2{\cdot}COOH \rightarrowtail C_6H_5{\cdot}CH_2 \,\vdots\, CH_2{\cdot}COOH \rightarrowtail C_6H_5{\cdot}COOH$$

(Phenylvaleric acid) (Phenylpropionic acid) (Benzoic acid)

Knoop indicated the probability of similar changes playing a part in the oxidations of the physiologically important normal fatty acids present in the animal body. Attention was drawn to the formation and excretion of β-hydroxybutyric acid and acetoacetic acid by diabetics, especially when large amounts of fats were being consumed (Geelmuyden, Magnus Levy, Waldvogel, Schwarz, and many others). Furthermore, the fact was recalled that normal men when on a diet containing little or no carbohydrate excreted the same acids, especially when the consumption of fats was large. It appeared not unlikely, therefore, that β-hydroxybutyric acid and acetoacetic acid were intermediate products in the β-oxidation of butyric acid or of higher fatty acids which might yield butyric acid on oxidation. Moreover, direct experiments upon normal dogs and upon human diabetics showed that an increased excretion of β-hydroxybutyric acid and acetoacetic acid (and acetone) may follow administration of salts of butyric acid (Blum, Schwarz):—

$$CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}COOH \rightarrowtail CH_3{\cdot}CHOH{\cdot}CH_2{\cdot}COOH \rightarrowtail CH_3{\cdot}CO{\cdot}CH_2{\cdot}COOH$$

(Butyric acid) (β-Hydroxybutyric acid) (Acetoacetic acid)

The theory of the degradation of fatty acids by loss of two carbon atoms at a time by oxidation in the β-position bore an interesting relation to the fact that fatty acids are contained in milk-fat having 18, 16, 14, 12, 10, 8, 6, and 4 carbon atoms, thus indicating the possibility of a progressive β-oxidation from higher fatty acid to lower. Fatty acids with an uneven number of carbon atoms are absent from most typical animal fats.

Notwithstanding the foregoing evidence pointing to the probability of the occurrence of β-oxidation, there was much opposition to the acceptance of this theory. The formation of β-hydroxybutyric acid and acetoacetic acid from fatty acids was ascribed by many to *synthesis* from simpler substances instead of to the β-oxidation of butyric acid. The objection was put forward that there was no purely chemical analogy for β-oxidation of saturated fatty acids. Friedmann wrote "From the standpoint of pure chemistry, the assumption of oxidation at the β-position is opposed to the facts that are known about the oxidation, substitution and condensation of fatty acids, since only

the hydrogen atoms attached to the α-carbon atom have been found to be capable of reaction and no observation is known showing that the hydrogen atoms attached to the β-carbon atoms are capable of undergoing reaction". Shortly before this Dakin was able to show that as a matter of fact an extraordinarily close pure chemical analogy did exist for Knoop's theory of biochemical oxidation.

While it is true that most of the laboratory oxidizing agents only act on fatty acids at high temperatures and that the products formed by these violent reactions have no biological significance[1] it was found that by suitable choice of an oxidizing agent entirely different results might be obtained. Thus it was found that butyric acid when neutralized and digested at 37° with hydrogen peroxide gave acetoacetic acid, acetone and other products, principally lower fatty acids and carbon dioxide. When the reaction was carried out at higher temperatures, the acetoacetic acid is converted into acetone with loss of carbon dioxide, according to the general reactions of β-ketonic acids. The yield of acetone may amount to as much as fifty per cent of the theoretical amount:—

$$\underset{\text{(Butyric acid)}}{CH_3\cdot CH_2\cdot CH_2\cdot COOH} \rightarrow \underset{\text{(Acetoacetic acid)}}{CH_3\cdot CO\cdot CH_2\cdot COOH} \rightarrow \underset{\text{(Acetone)}}{CH_3\cdot CO\cdot CH_3}$$

This reaction demonstrated the occurence of β-oxidation *in vitro* in the clearest fashion. It was found impossible to detect β-hydroxybutyric acid as an intermediate product of oxidation, although this substance is readily oxidized by hydrogen peroxide to acetoacetic acid, acetone, etc. Acetoacetic acid may be regarded as derived from the hypothetical di-hydroxybutyric acid by loss of a molecule of water.

This reaction with butyric acid was extended to higher fatty acids and it was found that every normal higher fatty acid when neutralized and warmed with hydrogen peroxide, gave the corresponding ketone containing one less carbon atom. The ketone must be assumed to be derived from a β-ketonic acid by loss of carbon dioxide. Stearic acid, for example, gave quindecyl-methyl-ketone: $CH_3\cdot(CH_2)_{14}\cdot CO\cdot CH_3$. Lower fatty acids are formed simultaneously by oxidation.

[1] In general, the oxidation of the higher fatty acids with the usual chemical reagents results principally in the production of dibasic acids. For example, myristic acid ($C_{14}H_{28}O_2$) on long-continued boiling with nitric acid (sp. gr. 1·3) yields varying proportions of suberic, pimelic, adipic, glutaric, succinic, and oxalic acids. Palmitic acid on oxidation with alkaline permanganate yields adipic, succinic, and oxalic acids together with caproic, butyric, and acetic acids. There is no reason to believe that the higher dibasic acids are intermediary products in the metabolism of fats.

Phenylpropionic acid which, according to Knoop, underwent β-oxidation in the body, with formation of benzoic acid, when oxidized with hydrogen peroxide gave benzoylacetic acid, acetophenone, and benzoic acid. Benzoylacetic acid and acetophenone were subsequently found to be intermediate products of the biochemical oxidation of phenylpropionic acid (Dakin):—

$$\underset{\text{(Phenylpropionic acid)}}{C_6H_5 \cdot CH_2 \cdot CH_2 \cdot COOH} \rightarrow \underset{\text{(Benzoylacetic acid)}}{C_6H_5 \cdot CO \cdot CH_2 \cdot COOH} \rightarrow \underset{\text{(Acetophenone)}}{C_6H_5 \cdot CO \cdot CH_3}$$

It was clear therefore that exception to Knoop's theory of β-oxidation could not be taken on the ground of lack of chemical analogy.

It should not be inferred however that hydrogen peroxide only attacks fatty acids in the β-position. α-Oxidation undoubtedly occurs to some extent and Cahen and Hurtley have demonstrated oxidation in the γ-position by obtaining succinic acid from butyric acid.

Formation in the Liver of Acetoacetic Acid from Higher Fatty Acids.—In the meantime Knoop's theory had led Embden and his coworkers to make experiments upon the catabolism of fatty acids which furnished striking confirmation of the theory of β-oxidation. They found that the small quantity of acetone normally formed during the perfusion of a freshly excised liver with defibrinated blood was greatly increased when certain amino or fatty acids were added to the blood prior to perfusion. The systematic examination of the fatty acids led to the following remarkable result. Of the normal fatty acids from butyric to decoic acid all those and only those with an even number of carbon atoms gave rise to a marked increase in acetone formation. Subsequent experiments showed that the acetone was derived from the decomposition of acetoacetic acid.[1]

	Normal Fatty Acid	Formation of Acetoacetic Acid
Formic acid	$H \cdot COOH$	—
Acetic acid	$CH_3 \cdot COOH$	variable
Propionic acid	$CH_3 \cdot CH_2 \cdot COOH$	—
Butyric acid	$CH_3 \cdot CH_2 \cdot CH_2 \cdot COOH$	+
Valeric acid	$CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH$	—
Caproic acid	$CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH$	+
Heptylic acid	$CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH$	—
Octoic acid	$CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH$	+
Nonoic acid	$CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH$	—
Decoic acid	$CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH$	+

[1] The later experiments of Blum, Dakin, Friedmann and Maase and Neubauer make it appear most probable that part of the acetoacetic acid was asymmetrically reduced to β-hydroxybutyric acid. Thus all of the so-called "acetone bodies" are undoubtedly formed from the catabolism of fatty acids in the liver.

The assumption that the normal fatty acids undergo oxidation with loss of two, or some multiple of two, carbon atoms at each successive step, furnishes a very satisfactory explanation of the widely different behaviour of the fatty acids with an even and an uneven number of carbon atoms as regards their ability to form acetoacetic acid. The oxidation of a normal acid with an even number of carbon atoms would eventually lead to a straight chain of four carbon atoms from which acetoacetic acid would be formed, whereas the acids with an uneven number of carbon atoms would yield chains of either three or five carbon atoms which obviously for structural reasons would not yield acetoacetic acid.

Acetoacetic acid is of course not to be regarded as an end-product of fatty acid catabolism. It is itself an intermediate product and is normally further oxidized to carbon dioxide and water. Fortunately for the success of the experimental method, acetoacetic acid appears to be relatively more stable than most other intermediate substances formed in fatty acid catabolism and hence lends itself more readily to isolation. Its ready conversion on heating into acetone which may be distilled off and easily estimated quantitatively are also important practical points.

The fate of acetic acid on perfusion through the surviving liver presents some peculiar and significant features. The early experiments by Friedmann showed no increase in acetoacetic acid—a result which might have been expected. Later experiments by A. Loeb on the other hand indicated that presence of acetates in the perfusion blood led to a very large increase in acetoacetic acid production. On further investigation Friedmann found that the divergent results were due to differences in experimental conditions for it was found that when the liver contained little glycogen much acetoacetic acid was formed whilst little or none was formed if much glycogen was present in the liver cells. It was next ascertained by Embden and Loeb that the inhibitory effect of glycogen was also exerted by salts of other acids such as propionic and *n*-valeric, while lactic acid was rather less effective. Formic acid which is oxidised with difficulty in the surviving liver had but little restraining action. It was found also that materially less acetic acid was decomposed in the livers containing much glycogen than when the latter substance was scarce.

The interpretation first placed on the inhibitory action of salts of other acids was naturally enough that decomposition of acetic acid in the liver was thereby diminished through the simultaneous, possibly preferential, oxidation of the other acids. But it is now generally

considered that the formation of acetoacetic acid from acetic acid in the liver is not an indirect reaction involving oxidation or reduction but apparently represents a direct condensation:

$$2CH_3 \cdot COOH \rightarrow CH_3 \cdot CO \cdot CH_2 \cdot COOH.$$

This reaction, if it really takes place as represented, and some reserve is certainly called for, is of extraordinary interest as illustrating the capacity of the liver cells for effecting synthesis. The result is the more remarkable when the special conditions are recalled which are requisite for the nearest analogous reaction *in vitro,* namely the condensation of dry ethyl acetate with metallic sodium or sodium ethylate. However, it will be recalled that the synthetic methods for coupling the amino acids are equally artificial and remote from those known to occur in the living cell.

In connection with the problem of acetoacetic acid formation from acetic acid in the liver, it was clearly desirable to examine possible oxidation products of acetic acid. Glycollic acid and glyoxylic acid according to Mochizuki yield no acetoacetic acid, while, according to Embden and Loeb, glycollic acid yields a little acetoacetic acid. They are inclined to regard the latter as possibly formed from acetic acid arising from the reduction of glycollic acid but this is by no means to be regarded as settled. Later experiments by Loeb gave positive results only when the concentration of glycollic acid was very high.

In connection with the inhibition of acetoacetic acid formation or antiketogenic action, as it is frequently called, it may be mentioned that most but not all (cp. phenylpyruvic acid) substances which have this effect are convertible in the liver either into glucose or lactic acid. The inhibitory effects of many substances on normal acetoacetic acid formation from fatty and aromatic acids has been studied by Embden and his colleagues.

Formation of Acetoacetic Acid from Fatty Acids in Diabetic Organisms.—The study of the fate of fatty acids, especially as regards their ability to yield β-hydroxybutyric acid, acetoacetic acid and acetone when fed to cases of human or experimental diabetes, has also given valuable results. Baer and Blum have been chiefly responsible for this work, which supplements and corroborates the results obtained by Embden's method. Thus administration of the salts of butyric acid and isovaleric acid led to an increased excretion of β-hydroxybutyric acid, acetoacetic acid and acetone, while propionic acid and normal valeric acid did not. Similar results have been obtained by Ringer using phlorhizinised dogs. Baer and Blum in addition experimented

with a number of acids with branched chains and also with amino acids. These results will be referred to later.

More recently Blum has shown that the administration of very large quantities of salts of butyric, caproic, or isovaleric acid to *normal* dogs is followed by the excretion of β-hydroxybutyric acid, acetoacetic acid, and acetone in the urine. Propionic and normal valeric acid, as expected, fail to yield these substances.

Formation of Glucose from Fatty Acids in Diabetic Organisms. A clue to the mechanism of the oxidation of normal fatty acids which contain an uneven number of carbon atoms and hence yield no acetoacetic acid in the liver has been furnished by Ringer. He found that propionic acid when given to a starving dog completely under the influence of phlorhizin was quantitatively converted into glucose. Earlier experiments by Höckendorf had shown that normal propyl alcohol which presumably gives propionic acid in the body, and other alcohols with an uneven number of carbon atoms, led to an increased glucose excretion. An indication of the probable course of the reaction has been recently obtained by Blum and Woringer who found both lactic and pyruvic acids in the urine of rabbits and dogs receiving large doses of propionic acid. The conversion of lactic acid quantitatively and pyruvic acid partially into glucose in the diabetic organism is well established, as is also the interconversion of the two acids. It is impossible at present to say whether lactic or pyruvic or possibly acrylic acid is the first product of oxidation. The reaction may be represented as follows:

$$CH_3 \cdot CH_2 \cdot COOH \rightarrow \left\{ \begin{array}{c} CH_3 \cdot CHOH \cdot COOH \\ \downarrow\uparrow \\ CH_3 \cdot CO \cdot COOH \end{array} \right\} \rightarrow \text{d-glucose.}$$

On experimenting with homologues of propionic acid Ringer and Jonas found that normal valeric and heptylic acids gave glucose about in proportion to the amount of propionic acid they might furnish through β-oxidation, while butyric and caproic acids gave no sugar but an increase in acetoacetic acid. The results may be tabulated as follows:

Normal Fatty Acid	Glucose Formation	Acetoacetic Acid Formation
Formic acid	—	—
Propionic acid	+	—
Butyric acid	—	+
Valeric acid	+	—
Caproic acid	—	+
Heptylic acid	+	—

These results taken together with those of Embden constitute very strong support for Knoop's theory of β-oxidation.

From the evidence presented it is inferred that normal saturated fatty acids and their phenyl derivatives at least in part undergo oxidation in such a fashion that they lose two terminal carbon atoms (or some multiple of two carbon atoms) at each successive step in their decomposition.

The Mechanism of β-Oxidation.

For many reasons it is unlikely that a single reaction is involved in the shortening of a fatty acid chain by two carbon atoms through a process of β-oxidation:—

$$R \cdot CH_2 \cdot \vdots\; CH_2 \cdot COOH \rightarrow R \cdot COOH$$

A complex oxidation of this character would necessitate a number of successive reactions, and it is obviously of great importance that this type of change should be accurately resolved into its simplest phases.

The excretion of *l*-β-hydroxybutyric acid following the administration of butyric acid to animals under certain conditions and the simultaneous presence of acetoacetic acid and acetone in the urine led to the belief that β-hydroxybutyric acid was the initial product of the oxidation of butyric acid and acetoacetic acid the second:—

$$CH_3 \cdot CH_2 \cdot CH_2 \cdot COOH \rightarrow CH_3 \cdot CHOH \cdot CH_2 \cdot COOH \rightarrow CH_3 \cdot CO \cdot CH_2 \cdot COOH$$

Embden's demonstration of the oxidation to acetoacetic acid of both butyric and β-hydroxybutyric acid when perfused through a surviving liver harmonized with this hypothesis.[1]

A case analogous to the formation of β-hydroxybutyric acid was furnished by Dakin's observation of the excretion of *l*-β-hydroxyphenylpropionic acid on administering phenylpropionic acid to cats. In addition to the hydroxy acid the corresponding ketonic acid, benzoylacetic acid, and acetophenone were also detected and the fact was further determined by Knoop and by Nencki that each of these intermediate substances when administered to an animal underwent oxidation with production of benzoic acid, i.e. the same end-product of oxidation as that obtained from phenylpropionic acid. The oxidation of phenylpropionic acid was reprensented as follows:—

[1] The oxidation of β-hydroxybutyric acid to acetoacetic acid was shown by Dakin and Wakeman to be due to an enzyme which could be roughly separated from liver tissue. The action of the enzyme was not very vigorous, but was markedly increased by the presence of oxyhæmoglobin. Oxyhæmoglobin alone was entirely without action.

(Phenylpropionic acid) $C_6H_5\cdot CH_2\cdot CH_2\cdot COOH$

$\downarrow$

(Phenyl-β-hydroxypropionic acid) $C_6H_5\cdot CHOH\cdot CH_2\cdot COOH$

$\downarrow$

(Benzoylacetic acid) $C_6H_5\cdot CO\cdot CH_2COOH \rightarrow C_6H_5\cdot CO\cdot CH_3$ (Acetophenone)

$\downarrow$

(Benzoic acid) $C_6H_5\cdot COOH$

From these results the inference was drawn that normal saturated fatty acids in general might undergo β-oxidation according to the following scheme:—

$$\begin{array}{c} R\cdot CH_2\cdot CH_2\cdot COOH \\ \downarrow \\ R\cdot CHOH\cdot CH_2\cdot COOH \\ \downarrow \\ R\cdot CO\cdot CH_2\cdot COOH \\ \downarrow \\ R\cdot COOH \end{array}$$

There are, however, certain difficulties in the assumption of a β-hydroxy acid as the first step in the normal catabolism of fatty acids and their aromatic derivatives, which call for consideration. Perhaps the least serious objection to the theory is the fact that the hydroxy acids in general appear to be much less readily oxidized in the body than the corresponding saturated or unsaturated acids.[1] From the purely chemical point of view it is certainly remarkable to find that in the animal body butyric acid is more readily oxidized than β-hydroxybutyric acid. With the usual laboratory oxidizing agents the hydroxy acids are infinitely more readily oxidized than the unsubstituted acids.

But it may be argued with some considerable justice that the results of the sudden administration of very large quantities of a substance may be entirely different from the results obtained when the substance is slowly formed in cells provided with a mechanism for its immediate transformation into other compounds. When large doses of substances are rapidly administered to an animal one has no proof that the bulk of the substance ever reaches the sphere normally concerned with its metabolism.

That β-hydroxybutyric acid certainly does not originate *exclusively* by the direct hydroxylation of butyric acid was shown almost simultaneously by Blum, Dakin, Friedmann and Maase, and by Neubauer. It was found that the liver was able to effect the asymmetric *re-*

[1] On administering 3·0 grms. β-phenyl-β-hydroxypropionic acid as sodium salt to a 3 kg. cat, 2·35 grms. were recovered unchanged. No unoxidized β-phenylpropionic acid could be found after injection of an equal quantity of this acid.

duction of acetoacetic acid to *l*-β-hydroxybutyric acid.[1] Von Lagermark has found the same enzyme or keto-reductase in the muscle and kidney of the dog. It is absent from blood, lung, pancreas and spleen. This reduction is the exact reverse of the oxidation of β-hydroxybutyric acid by the enzyme previously referred to. The liver is thus provided with a mechanism, dependent upon the antagonistic action of two ferments, by which the mutual interconversion of β-hydroxybutyric acid and acetoacetic acid may be effected. The one ferment action is an oxidation dependent upon the presence of free oxygen or oxyhæmoglobin, while the other ferment action is a reduction. The source of the two hydrogen atoms necessary for this reduction is unknown.[2]

$$CH_3\cdot CHOH\cdot CH_2\cdot COOH \underset{\text{oxidation}}{\overset{\text{reduction}}{\rightleftarrows}} CH_3\cdot CO\cdot CH_2\cdot COOH$$

Little is known of the conditions determining whether oxidation or reduction shall predominate and doubtless we have here to deal with a delicately adjusted equilibrium. Under ordinary conditions a normal minced dog's liver is more active in reducing acetoacetic acid than in oxidizing β-hydroxybutyric acid, but these observations are of little value in judging of the reactions during life. It is probable that the presence of readily oxidizable substances (e.g. carbohydrates) in the liver would influence the balance markedly, and in this connexion the influence of carbohydrates in diminishing the acidosis of diabetes may be recalled (Hirschfeld, Rosenfeld). Blum and Nakano find that when sodium hydroxybutyrate is administered to rabbit, dog or man under normal conditions the excretion of acetoacetic acid is slight, but when the normal metabolic functions of the liver are disturbed by chloroform anæsthesia much acetoacetic acid is found in the urine. They have also made the unexpected observation that sodium hydroxybutyrate when given with sodium chloride solution leads to a much smaller acetoacetic acid production than when large quantities of glucose are administered. Usually glucose has the opposite or 'antiketogenic' effect. The interconversion of hydroxybutyric and acetoacetic acids has also been investigated by Pribram

[1] Benzoylacetic acid is similarly reduced to *l*-β-phenyl-β-hydroxypropionic acid (Friedmann, Dakin).

[2] It is possible that a β-ketonic acid might undergo a type of Cannizzaro reaction by which one molecule of the ketonic acid would be reduced to the β-hydroxyy acid, while a second would undergo further oxidation. Thus, the body possesses catalysts capable of converting benzaldehyde (2 mols.) into benzyl alcohol (1 mol.) and benzoic acid (1 mol.).

and by Marriott. The latter assumes that the optically inactive hydroxy acid is produced by reduction of acetoacetic acid and that the dextro component is then preferentially burned, but the facts observed so far do not appear to require so unusual an hypothesis. The relation existing between the amount of β-hydroxybutyric and acetoacetic acids excreted in human diabetes and similar conditions has been investigated by Kennaway. It is found that when the total daily excretion of the two acids exceeds two and one half grams the proportion of hydroxy acid is two to five times that of the ketonic acid. The ratio of the amounts of the two acids is clearly greatly influenced by the total concentration and the condition of the organism under examination.

There is still another way by which β-hydroxy acids may arise in the body apart from the direct hydroxylation of fatty acids or the reduction of ketonic acids. It has been found that the unsaturated acids may take up the elements of water with formation of β-hydroxy acids. Thus, cinnamic acid may yield phenyl-β-hydroxypropionic acid (Dakin). The reaction is a reversible one:—

$$\underset{\text{(Cinnamic acid)}}{C_6H_5\cdot CH{=}CH\cdot COOH} \rightleftharpoons \underset{\text{(Phenyl-}\beta\text{-hydroxypropionic acid)}}{C_6H_5\cdot CHOH\cdot CH_2\cdot COOH}$$

It is manifest therefore that *the detection of a β-hydroxy acid as an intermediate product of the oxidation of a normal fatty acid cannot in itself be regarded as convincing proof of its formation by direct oxidation of the fatty acid in a single step. On the other hand, the possibility of the direct oxidation of saturated fatty acids with formation of β-hydroxy acids has not been disproved.*

Blum has advanced the view that the normal path for the catabolism of butyric acid is by way of acetoacetic acid without intermediate formation of β-hydroxybutyric acid. The latter substance, when formed, is assumed to be derived exclusively from the reduction of acetoacetic acid and not by the direct oxidation of butyric acid. Blum assumes, moreover, that β-hydroxybutyric acid does not normally undergo oxidation in the body with formation of acetoacetic acid but is decomposed in some other way. The actual demonstration of the formation of acetoacetic acid from β-hydroxybutyric acid by Embden and by Wakeman and Dakin is referred by Blum to a pathological condition of the liver cells. The evidence adduced by Blum in support of his contention that β-hydroxybutyric acid is not an intermediate step in the oxidation of butyric acid to acetoacetic acid is mainly based upon the fact that he failed to produce a marked acetonuria by the subcutaneous administration of sodium β-hydroxy-

butyrate to a dog which under similar conditions developed acetonuria when sodium butyrate or sodium acetoacetate was administered.

It may be fairly questioned whether these results really justify the interpretation placed upon them by Blum. When it is realized that the increase in the amount of acetone in the urine following the administration of 17 grms. of sodium butyrate only corresponds to about one-half per cent. of the butyric acid, while the acetone found following the giving of 10 grms. of sodium acetoacetate itself only corresponds to a little over 3 per cent of the acetoacetic acid, it is obvious that much acetoacetic acid might have been formed from the β-hydroxybutyric acid without materially increasing the acetoacetic acid and acetone of the urine.[1]

Moreover, other workers have apparently demonstrated the formation of acetoacetic acid from β-hydroxybutyric acid in the intact animal. Thus McKenzie found acetoacetic acid and acetone in the urines of dogs that had received injections of inactive sodium β-hydroxybutyrate. Minkowski gave 10 grms. of sodium l-β-hydroxybutyrate to a depancreatized dog and observed the excretion of acetoacetic acid and acetone, while Magnus Levy obtained similar results with a dog rendered diabetic by phlorizin.

From the results of experiments with butyric acid and phenylpropionic acid it can hardly be doubted that the oxidation, at least in the case of these acids, takes place with formation of a β-ketonic acid, and that the β-hydroxy acids are in part at least secondary products of the reduction of the ketonic acid. The results may be represented as follows:—

$$
\begin{tikzcd}
 & R\cdot CH_2\cdot CH_2\cdot COOH \arrow[dl] \arrow[dr] & \\
R\cdot CO\cdot CH_2\cdot COOH \arrow[rr, shift left] \arrow[d] & & R\cdot CHOH\cdot CH_2\cdot COOH \arrow[ll, shift left] \\
R\cdot COOH & &
\end{tikzcd}
$$

The question arises as to whether the foregoing scheme represents the mechanism of β-oxidation not only of the simple acids above

[1] It is frequently very difficult to produce an excretion of acetoacetic acid by the intact animal through the administration of substances which are believed to yield acetoacetic acid as products of their catabolism. The writer has frequently given phenylalanine and tyrosine to animals so that much appeared unchanged in the urine, without detecting acetoacetic acid in the urine. On perfusing the surviving liver with these amino acids, acetoacetic acid is formed in abundance.

referred to but also in the case of the higher fatty acids. Judging simply by chemical analogy it would be expected that a fatty acid such as stearic acid might undergo the same type of oxidation as butyric acid. But it must be admitted that no β-ketonic acids other than acetoacetic and benzoylacetic acid have been detected among the products of normal fatty acid catabolism in the animal body.[1] On the other hand unsaturated hydroxy acids of the type of ricinoleic acid are known and these acids are isomeric with the ketonic acids. Moreover, normal ketonic fatty acids have been found in vegetable organisms, and it is not unlikely that the naturally occurring aliphatic ketones found in essential oils such as methyl-n-nonyl ketone, methyl-n-heptyl ketone and methyl-n-amyl ketone are formed from the β-ketonic acids derived from lauric, capric, and caprylic acids (Dakin). Bougault and Charaux have recently shown that fungi of genus Lactarius contain an acid named lactarinic acid together with stearic acid. Lactarinic acid was shown to be 6-ketostearic acid— $CH_3 \cdot (CH_2)_{11} \cdot CO \cdot (CH_2)_4 \cdot COOH$. Whether lactarinic acid arises from the biochemical oxidation of stearic acid is not as yet known.

It would appear, therefore, that β-oxidation of normal saturated acids with intermediate formation of β-ketonic acids does occur in the oxidation of butyric and phenylpropionic acids (i.e. the simplest acids of the type $R \cdot CH_2 \cdot CH_2 \cdot COOH$ in which such a reaction is possible), but the question whether β-ketonic acids are formed as initial stages in the oxidation of the higher fatty acids is at present to be regarded as possible but not proven.

There is another group of substances, other than the β-hydroxy and β-ketonic acids, which must be considered as possible oxidation products of the fatty acids, namely the unsaturated acids. A few years ago the possibility for an unsaturated acid to arise from the direct oxidation of a saturated fatty acid seemed remote largely because no closely analogous reaction was known to take place *in vitro*. But the reason for this was largely due to the fact that the unsaturated acids are so much more readily oxidised than the saturated acids by most of the ordinary oxidising agents used by the organic chemist,

[1] Blum and Koppel have described the formation of methyl-propyl-ketone from the oxidation of diethylacetic acid in the animal body. The ketone is doubtless derived from a β-ketonic acid:—

$$CH_3 \cdot CH_2 \cdot \underset{\displaystyle C_2H_5}{\underset{|}{CH}} \cdot COOH \longrightarrow CH_3 \cdot CO \cdot \underset{\displaystyle C_2H_5}{\underset{|}{CH}} \cdot COOH \longrightarrow CH_3 \cdot CO \cdot CH_2 \cdot C_2H_5$$

that even if formed as intermediate products they would be at once further oxidised. It will be recalled that a common test for distinguishing saturated from unsaturated substances is to add dilute potassium permanganate to the compound dissolved in sodium carbonate solution when immediate reduction of the permanganate takes place in the presence of unsaturated compounds. But, in the animal organism, this marked difference does not seem to exist and indeed in many cases the saturated acid seems to be more susceptible to oxidation than the unsaturated one.

The direct formation of an unsaturated acid from a saturated one requires the removal of two atoms of hydrogen. Such a reaction to-day appears much less extraordinary since the clear proof afforded by Wieland that many oxidations involve not an activation of oxygen but rather of hydrogen which is then taken up by a suitable acceptor or catalyst (see p. 12). Wieland's theory of dehydrogenation fits in admirably with the idea that unsaturated acids may be formed by the oxidation of saturated ones, although directly analogous reactions have not yet been carried out in the laboratory. The change appears, however, to have been shown to occur in the body. Battelli and Stern in 1910 showed that some animal tissues, especially muscle, in contact with oxygen, could rapidly effect the oxidation of succinic acid. They identified malic acid as a product of the reaction. Later investigations by Einbeck have shown with a considerable degree of certainty that fumaric acid is first produced and that this acid secondarily takes up water with formation of malic acid The production of fumaric acid appears to be quantitative, while its subsequent conversion into malic acid takes place to the extent of about seventy-five per cent. The reaction may be represented as follows:

$$COOH \cdot CH_2 \cdot CH_2 \cdot COOH \rightarrow COOH \cdot CH{=}CH \cdot COOH \rightleftharpoons COOH \cdot CHOH \cdot CH_2 \cdot COOH$$

The ease with which succinic acid is oxidised by muscle tissue is the more remarkable when its great resistance to ordinary chemical oxidising reagents is considered.

There are at least two other cases in which the formation of an unsaturated acid by the oxidation of a saturated acid has been demonstrated. Dakin found cinnamoylglycine in the urines of cats which had received injections of the salts of phenylpropionic acid and phenylvaleric acid and other related substances, and Sasaki found the glycine derivative of furfuracrylic acid in the urine of animals which had been given furfurpropionic acid:—

$$R \cdot CH_2 \cdot CH_2 \cdot COOH \rightarrow R \cdot CH{=}CH \cdot COOH$$

Leathes and Meyer Wedell have shown that on feeding animals with oils or fats containing unsaturated acids, fats accumulate in the liver, the fatty acids of which, judged by their iodine values, are even more unsaturated than those contained in the food. But there are two possible explanations of this phenomenon. Either new double linkages are introduced into the fatty acid molecule by oxidation, or structurally isomeric unsaturated acids which absorb iodine more readily are formed by intramolecular rearrangement.

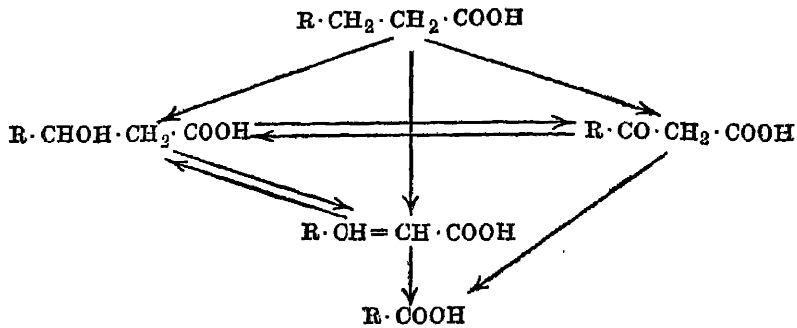
It is certain that unsaturated fatty acids are formed in the body by the oxidation of saturated acids, but we have not at present any means of knowing whether this change is ordinarily effected by a single process of oxidation. Thus, although cinnamic acid is unquestionably formed from phenylpropionic acid in the animal body, it may originate indirectly from phenyl-β-hydroxypropionic acid or benzoylacetic acid, both of which substances are intermediary products of the catabolism of phenylpropionic acid (cf. diagram, p. 42).

In general it may be said that unsaturated acids are formed in the animal body during the catabolism of saturated fatty acids; that α, β-unsaturated acids may be formed indirectly from saturated acids through the intermediate formation of β-hydroxy and β-ketonic acids; and that the direct oxidation of saturated to unsaturated acids without intermediate formation of substitution derivatives occurs in some cases.

Assuming that the α, β-unsaturated acids, however formed, do represent an intermediate stage in the β-oxidation of saturated fatty acids, their further oxidation with loss of two carbon atoms is readily understood:—

$$R \cdot CH = CH \cdot COOH \rightarrow R \cdot COOH$$

Chemical analogies for such an oxidation abound, but in the case of the biochemical oxidation it appears that the reaction is not always as simple as might appear. It is known that the unsaturated acids may by reversible reactions take up water and pass over into β-hydroxy acids and in turn yield β-ketonic acids, so that it is not certain that the oxidation of unsaturated acids to saturated acids with two fewer carbon atoms takes place *directly* without intermediate formation of β-substitution derivatives. The various changes which are believed to be mainly concerned with β-oxidation, including oxidation and reduction, hydration and dehydration, most of them reversible reactions, are shown diagrammatically as follows:—



Hydrolysis and Oxidation of β-Ketonic Acids.—It will be noted that no reference has as yet been made to the fate of the two carbon atoms removed from saturated fatty acids at each successive β-oxidation of the higher normal fatty acids. It might be imagined that formic acid could be an intermediate step in their conversion into carbon dioxide, but there is no satisfactory evidence in favour of this belief.

$$R \cdot CH_2 \mid CH_2 \cdot COOH \rightarrow R \cdot COOH + HCOOH + CO_2$$

Attempts that have been made to demonstrate the production of formic acid as the result of the oxidation of higher fatty acids have not given decisive results. Dakin and Wakeman have found no increase of formic acid in the blood used for perfusing livers in which the oxidation of higher fatty acids has been in progress (unpublished results). An alternative decomposition on the lines of the well known "acid hydrolysis" of β-ketonic esters would be expected to give acetic acid:

$$R \cdot CH_2 \cdot CO \cdot CH_2 \cdot COOH \rightarrow R \cdot CH_2 \cdot COOH + CH_3 \cdot COOH$$

Attempts to demonstrate such a formation of acetic acid by Embden and Michaud and others have not been successful. Notwithstanding this, Friedmann has assumed that acetic acid must necessarily be produced. Since acetic acid is now known to be capable of acetoacetic acid formation Friedmann rejects the whole theory of β-oxidation on account of the failure of all higher fatty acids, including those with an odd number of carbon atoms, to furnish acetoacetic acid via acetic acid. Such a circular process of reasoning does not merit very serious consideration. But further information as to the fate of the paired carbon groupings set free from fatty acids by biochemical oxydation is urgently needed.

There still remains the possibility of the decomposition of β-ketonic acids by oxidation. Little work has been done on this subject although they are known to be readily oxidised. Emmerling and Oppenheim

oxidised acetoacetic ester with potassium permanganate and obtained acetic and oxalic acids, but this result is of limited biological significance. The writer has oxidised sodium acetoacetate with hydrogen peroxide, a reagent which frequently simulates biochemical reactions. The reaction takes place readily at room temperature with formation of acetic, glyoxylic and formic acids and carbon dioxide. The changes may be represented as follows:

$$\begin{array}{lcllcl} CH_3 & & CH_3 & & & \\ | & & | & & & \\ CO & & COOH & & & \\ | & \longrightarrow & & & & \\ CH_2 & & CHO & & & \\ | & & | & \longrightarrow & H\cdot COOH + CO_2 & \\ COOH & & COOH & & & \end{array}$$

It is conceivable that an analogous decomposition may take place in the body but it has not yet been demonstrated.

Ketone Formation from β-Ketonic Acids.—Acetoacetic acid is found in the urine of animals in an advanced stage of diabetes; in normal animals after administering very large amounts of substances which yield acetoacetic acid on oxidation; and also in man when carbohydrates are omitted from the diet and fat catabolism is much increased. The acetoacetic acid is commonly accompanied by more or less acetone. Until the recent discovery of good methods for the separate determination of acetoacetic acid and acetone (Folin, Embden), small amounts of acetoacetic acid were commonly mistaken for acetone into which it passed with ease. Acetone was therefore regarded as one of the first steps in the decomposition of acetoacetic acid in the body. Similarly acetophenone was found to accompany benzoylacetic acid when the latter substance was detected in the urine of animals receiving aromatic acids, such as phenylpropionic acid and phenylvaleric acid:—

$$CH_3\cdot CO\cdot CH_2\cdot COOH \longrightarrow CH_3\cdot CO\cdot CH_3 + CO_2$$
$$C_6H_5\cdot CO\cdot CH_2\cdot COOH \longrightarrow C_6H_5\cdot CO\cdot CH_3 + CO_2$$

Does this decomposition of β-ketonic acids with formation of ketones represent the main normal course of their catabolism? There is a considerable amount of evidence against this supposition.

In the first place, Geelmuyden has shown that acetone is oxidized in the animal body with very considerable difficulty. Similarly, acetophenone is not very easily oxidized in the animal body and moreover is distinctly more toxic than are the acids which yield it on oxidation.[1]

[1] Benzoic acid is the end-product of the oxidation of acetophenone in the body, but under certain conditions part of the acetophenone may undergo reduction to phenyl-methyl-carbinol, which is excreted in combination with glucuronic acid.

Experiments made upon the oxidation of phenylbutyric acid and phenylvaleric acid have a direct bearing upon the question of ketone formation and prove that at least in the case of these acids, ketones, derived from the corresponding β-ketonic acids, are *not* formed. The evidence for this is as follows: phenylbutyric acid on oxidation in the body gives phenaceturic acid and no hippuric acid (Knoop); phenylacetone, the corresponding ketone, on the other hand, gives hippuric acid but no phenaceturic acid (Dakin); phenylvaleric acid gives on oxidation in the body hippuric acid (Knoop); while benzylacetone, the corresponding ketone, gives phenaceturic acid (Dakin). Analogous experiments by Herrmanns have led to similar results.

It appears legitimate to transfer the results obtained with phenylvaleric acid to the higher fatty acids and to conclude that if the higher fatty acids in undergoing β-oxidation do yield β-ketonic acids, these acids are not converted into the corresponding ketones by loss of carbon dioxide.

	Substance.	Oxidation Product Excreted in Combination with Glycine.
Acid	$C_6H_5 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH$	$C_6H_5 \cdot CH_2 \cdot COOH$
Ketone	$C_6H_5 \cdot CH_2 \cdot CO \cdot CH_3$	$C_6H_5 \cdot COOH$
Acid	$C_6H_5 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH$	$C_6H_5 \cdot COOH$
Ketone	$C_6H_5 \cdot CH_2 \cdot CH_2 \cdot CO \cdot CH_3$	$C_6H_5 \cdot CH_2 \cdot COOH$

It is probably safe to assume that acetone and acetophenone are not normal steps in the catabolism of butyric and phenylpropionic acids, but that, under certain conditions, the acetoacetic acid and benzoylacetic acid formed from these acids escape complete oxidation and may slowly pass over into the ketones.

Propionic, Acetic and Formic Acids. — In the introduction to this chapter reference has been made to the ease with which salts of these acids undergo practically complete oxidation in the animal body. Almost nothing is known of the mechanism of this process of oxidation. Acetic and formic acids obviously cannot undergo β-oxidation as the higher fatty acids do, and there is no good evidence to prove that propionic acid undergoes this type of change. On the other hand, it is possible that propionic acid undergoes α-oxidation in the animal body since both lactic and pyruvic acids have been found as intermediate products by Blum and Woringer. Since both lactic and propionic acids yield glucose quantitatively in the diabetic organism, it may be inferred that oxidation of the hydrogen attached to the α-carbon atom is the first step in the oxidation. It is however not unlikely that acrylic acid, which also yields glucose in the diabetic animal, may be the first product of oxidation and subsequently yields lactic acid.

The chemistry of the oxidation of acetic and formic acids is of special importance since these acids are constantly excreted in small amounts in the urine and it appears not improbable that these acids may be formed in the body as intermediate products of oxidation in large amounts, of which only a very small proportion escapes complete oxidation and is excreted. As to the mechanism of the oxidation of these acids, there is not much to be said. Formic acid is presumably directly oxidized to carbon dioxide[1], but in the case of acetic acid the question of intermediate products arises. Moderate amounts of acetic acid are completely burned in the body. Oxalic acid is not found in the urine following acetate administration, so that this substance, which is relatively resistant to further oxidation in the body, is not an intermediate step.[2] It would seem more probable that glyoxylic and formic acids might be intermediate products in the change. This reaction is readily brought about outside the body by oxidation with hydrogen peroxide (Hopkins). The only experimental evidence in support of this hypothesis is the fact that, under certain conditions, Wakeman and Dakin found an increased formic acid excretion to follow intravenous injection of sodium acetate. The increase, however, is very small and the results are not constant. Further investigation is much needed.

Other Types of Oxidation. — Satisfactory evidence has been adduced showing that the higher normal fatty acids undergo β-oxidation and that, in the case of the oxidation of butyric acid and phenylpropionic acid, the intermediate formation of β-hydroxy and β-ketonic acids has been determined. We have now to consider the question whether oxidation of saturated normal fatty acids may not take place in other ways. Although it is by no means improbable that other types of reaction than that of β-oxidation will be found to occur, so far no other has been observed.

Oxidation of normal saturated fatty acids in the α-position (except in the case of acetic and propionic acids) is excluded for many reasons: (1) Phenylpropionic acid is oxidized in the body only so far as benzoic acid, the aromatic ring remaining intact, while phenyl α-hydroxypropionic acid, $C_6H_5 \cdot CH_2 \cdot CHOH \cdot COOH$, and phenylpyruvic acid, $C_6H_5 \cdot CH_2 \cdot CO \cdot COOH$, substances which would be formed by α-oxidation of phenylpropionic acid, are completely oxidized in the

[1] Batteli and Stern have shown that many tissues, especially the liver, contain peroxidases which, in the presence of hydrogen peroxide, readily effect the oxidation of formic acid to carbon dioxide.

[2] Acetic acid is readily oxidized to oxalic acid by alkaline permanganate.

body, the aromatic nucleus being destroyed. (2) α-oxidation of normal higher fatty acids with an uneven number of carbon atoms would presumably yield acids with an even number of carbon atoms, which in turn would yield acetoacetic acid when perfused through a surviving liver, according to Embden's method. Acetoacetic acid formation from normal acids with an uneven number of carbon atoms does not occur, however. (3) Levulinic acid, $CH_3 \cdot CO \cdot CH_2 \cdot CH_2 \cdot COOH$, when administered to a diabetic animal, does not materially increase the excretion of acetoacetic acid as would be the case had α-oxidation occurred (Weintraud).

From the above and other reasons it is concluded that α-oxidation of normal saturated fatty acids containing more than three carbon atoms does not take place in the animal body, or is at least unusual.

The occurence of γ-oxidation of normal fatty acids is very unlikely, for in the first place γ-hydroxy acids and their lactones and γ-ketonic acids, such as levulinic acid, phenyl-γ-hydroxybutyric acid and phenyl-γ-hydroxyvaleric acid, are rather resistant to oxidation in the animal body; secondly, γ-oxidation, if it occured, would result in butyric acid and hence acetoacetic acid formation from acids with an uneven number of carbon atoms, such as heptylic acid, when given to diabetic animals, or perfused through a surviving liver. This acetoacetic acid formation, however, is not observed, hence it appears safe to conclude that oxidation of saturated normal fatty acids in the γ-position does not take place in the animal body.

That oxidation in the δ-position may occur is more probable than either the α or γ-position, although the evidence at present is against the assumption of its occurence. As previously mentioned, phenylvaleric acid is oxidized in the animal body to benzoic acid, which is excreted as hippuric acid (Knoop). This reaction obviously involves oxidation of the hydrogen attached to the δ-carbon atom, but Dakin showed, through the isolation of intermediate products, that the oxidation was a complex one involving two successive β-oxidations. There was no evidence that δ-oxidation as such had occurred. The following intermediate products of catabolism were identified: β-phenyl-β-hydroxypropionic acid, cinnamoylglycine, benzoylacetic acid and acetophenone. These are the same substances as are formed among the products of catabolism of phenylpropionic acid. Hence it is reasonable to assume that the phenylvaleric acid underwent β-oxidation yielding phenylpropionic acid, which in turn underwent β-oxidation again yielding benzoic acid:—

$$C_6H_5 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH \rightarrow C_6H_5 \cdot CH_2 \cdot CH_2 \cdot COOH \rightarrow C_6H_5 \cdot COOH$$

This type of reaction may be termed successive β-oxidation and is probably typical of the normal catabolism of straight chain saturated fatty acids. The actual mode of conversion of phenylvaleric acid into phenylpropionic acid could not be exactly determined, but it was shown that the corresponding β-hydroxy acid and α-β-unsaturated acid gave the same catabolic products as phenylvaleric acid:—

$$C_6H_5\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH_2\cdot COOH$$

$$\swarrow \qquad\qquad \searrow$$

$$C_6H_5\cdot CH_2\cdot CH_2\cdot CHOH\cdot CH_2\cdot COOH \rightleftharpoons C_6H_5\cdot CH_2\cdot CH_2\cdot CH = CH\cdot COOH$$

$$\searrow \qquad\qquad \swarrow$$

$$C_6H_5\cdot CH_2\cdot CH_2\cdot COOH$$

Finally reference must be made to the fate of δ-benzyllevulinic acid, $C_6H_5\cdot CH_2\cdot CH_2\cdot CO\cdot CH_2\cdot CH_2\cdot COOH$ investigated by Knoop and Oeser. Had γ-oxidation taken place at the ketone group phenylpropionic acid would have been formed which in turn gives benzoic (hippuric) acid in the body. Actually phenylacetic (phenaceturic acid) was obtained as the main product, a result which Knoop and Oeser interpret as due to reduction of the ketonic acid to phenylcaproic acid which then undergoes repeated β-oxidation. Phenyl-α-hydroxybutyric acid was isolated in smaller amount. Knoop and Oeser regard this experiment as definitely proving the possibility of the reduction of ketonic acids to saturated acids in the animal body. While this change in all probability can be accomplished in the body, it does not appear to the writer that the above experiment furnishes adequate proof. The formation of phenylacetic acid could equally well be accounted for by direct oxidation at the double linkage of one of the tautomeric forms of the acid, such as: $C_6H_5\cdot CH_2\cdot CH = COH\cdot CH_2\cdot CH_2\cdot COOH$.

II. THE UNSATURATED ACIDS.

The higher unsaturated acids are very important constituen of the animal body and their oxidation furnishes a readily availabl source of energy. Most chemical reagents attack the unsaturate acids infinitely more readily than they do the saturated acids, bu in the animal organism this difference is not nearly so marked. Thu phenylpropiolic acid, $C_6H_5 \cdot C \equiv C \cdot COOH$, is oxidized in the body wit much greater difficulty than either cinnamic, $C_6H_5 \cdot CH = CH \cdot COOH$ or phenylpropionic acid, $C_6H_5 \cdot CH_2 \cdot CH_2 \cdot COOH$ (unpublished obser vations). Of the three acids, the saturated phenylpropionic is oxidize in the body with greater ease than either of the others, but toward most chemical oxidizing agents it is by far the most resistant.

The question of the formation of unsaturated acids from saturate acids has been dealt with on p. 40, and we are now concerned simpl with their further decomposition. Oleic acid, $C_{18}H_{34}O_2$, which ma occur in structurally isomeric forms, and the still more highly un saturated acids related to it, are the most important members o this group. Unfortunately we know almost nothing of the biochemica oxidation of these acids and can only speculate as to the mechanism of their catabolism by analogy with other acids.[1]

The simplest unsaturated acid, acrylic acid, $CH_2 = CH \cdot COOH$ readily undergoes complete oxidation in the animal body withou yielding any clue to intermediate products (Luzatto). Recently Schwenken has shown that in the diabetic organism it gives glucose apparently completely. Hydracrylic acid or lactic acid are probabl intermediate products. Its phenyl derivative, cinnamic acid, $C_6H_5 \cdot CH = CH \cdot COOH$, when administered to animals has been found to underg decomposition in an interesting fashion and the results may hav a bearing upon the catabolism of other unsaturated acids. It had been observed long ago by Erdmann and Marchand that cinnami acid was oxidized in the body to benzoic acid which was excreted in the form of hippuric acid. By administering larger amounts o

[1] For information concerning the oxidation *in vitro* of the chief un saturated acids, consult "The Fats", by J. B. Leathes, this series, 1910.

ammonium cinnamate subcutaneously to cats and dogs (0·25—0·45 grm. per kilo) Dakin observed the excretion of laevo-β-phenyl-β-hydroxypropionic acid and acetophenone, the latter substance being derived from benzoylacetic acid. This formation of a β-hydroxy acid from the corresponding unsaturated acid by union with the elements of water, appears to be a reversible reaction, since excretion of cinnamoylglycine was observed to follow the administration of phenyl-β-hydroxypropionic acid.[1] The changes may be represented as follows:—

$$C_6H_5{\cdot}CH{=}CH{\cdot}COOH \rightleftharpoons C_6H_5{\cdot}CHOH{\cdot}CH_2{\cdot}COOH \rightleftharpoons C_6H_5{\cdot}CO{\cdot}CH_2{\cdot}COOH$$

The oxidation of cinnamic acid and phenyl-β-hydroxypropionic acid to benzoic acid may take place in several ways. The finding of benzoylacetic acid shows that at least part of the cinnamic acid was converted into benzoic acid through the formation of this substance as an intermediate product. Expressing the reaction in general terms one may therefore represent an α-β-unsaturated acid undergoing decomposition in the following fashion:—

$$R{\cdot}CH{=}CH{\cdot}COOH \rightarrowtail R{\cdot}CHOH{\cdot}CH_2{\cdot}COOH \rightarrowtail R{\cdot}CO{\cdot}CH_2{\cdot}COOH \rightarrowtail R{\cdot}COOH$$

It will be noticed that these changes have much in common with those representing the β-oxidation of a saturated fatty acid, such as butyric acid, or phenylpropionic acid. But, at present at any rate, there is no reason for assuming that an unsaturated acid, such as cinnamic acid, can undergo oxidation only by conversion into a β-hydroxy acid and β-ketonic acid. It seems reasonable to suppose that some of the unsaturated acids may undergo direct oxidation without previously taking up the elements of water.

Within recent years two excellent examples of the formation of β-hydroxy-acids from unsaturated acids have come to light. Friedmann and Maase have shown that crotonic acid on digestion with liver tissue is converted partially into *l*-β-hydroxybutyric acid:

$$CH_3{\cdot}CH{=}CH{\cdot}COOH \rightarrow CH_3{\cdot}CHOH{\cdot}CH_2{\cdot}COOH$$

Friedmann has made the curious observation that this reaction only takes place in the presence of air and, if the reaction vessel is filled with carbon dioxide, hydrogen or nitrogen, the change does not occur. On the other hand, the presence of excess of oxygen does not

[1] The conversion of a β-hydroxy acid into an unsaturated acid is often observed. Thus phenyl-β-hydroxypropionic acid readily gives cinnamic acid when warmed with hydrochloric acid. The reverse change, i. e. the formation of a β-hydroxy acid from an α-β-unsaturated acid is much less common. The conversion of fumaric acid into malic acid on prolonged heating with caustic soda solution is an example of this change.

4

accelerate the reaction and it is improbable that oxygen as such takes part directly in the change. The other example of hydroxy-acid formation from an unsaturated acid is due to Einbeck who found that fumaric acid when digested with liver tissue gives malic acid:

$$COOH \cdot CH = CH \cdot COOH \rightleftharpoons COOH \cdot CHOH \cdot CH_2 \cdot COOH$$

The reaction appears to be a balanced one and comes to an equilibrium when about seventy five per cent of malic acid has been formed.

When an unsaturated acid is carefully oxidized with a reagent such as potassium permanganate, as a general rule a dihydroxy acid is formed as the first product of oxidation, while on further oxidation the original carbon chain is broken at the double linkage with formation of a lower fatty acid. Thus cinnamic acid yields phenylglyceric acid and then benzoic acid:—

$$C_6H_5 \cdot CH = CH \cdot COOH \rightarrow C_6H_5 \cdot CHOH \cdot CHOH \cdot COOH \rightarrow C_6H_5 \cdot COOH$$

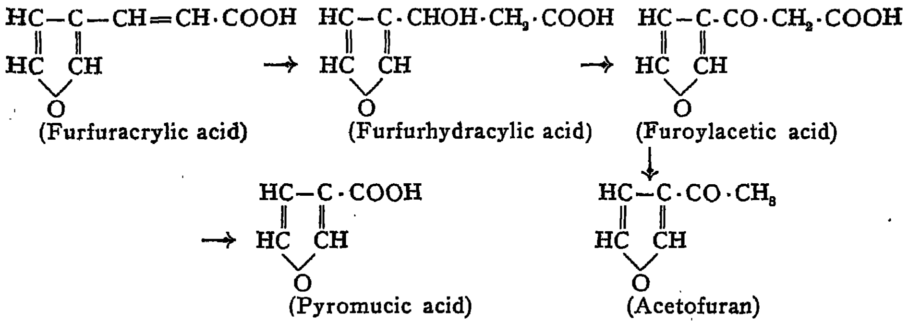
The question arises whether these dihydroxy acids are products of the oxidation of unsaturated acids in the body. There seems to be good evidence that they are *not* formed, at any rate to any large extent. Thus (1) it is found that phenylglyceric acid is oxidized in the body to hippuric acid only with great difficulty compared with cinnamic acid (Dakin). (2) Friedmann showed that the behaviour of crotonic acid, $CH_3 \cdot CH : CH \cdot COOH$, when perfused through a surviving liver, was quite different from that of α-β-dihydroxybutyric acid, $CH_3 \cdot CHOH \cdot CHOH \cdot COOH$ (see later). (3) Phenyl-β-γ-dihydroxybutyric acid $C_6H_5 \cdot CHOH \cdot CHOH \cdot CH_2 \cdot COOH$ is not a product of the oxidation in the animal body of phenylisocrotonic acid, $C_6H_5 \cdot CH : CH \cdot CH_2 \cdot COOH$, since the end-product of the oxidation of the latter substance is phenylacetic acid (Knoop), while the former yields benzoic acid (Dakin).

It appears, therefore, that the dihydroxy acids do not represent intermediate stages in the normal catabolism of singly unsaturated acids. It is quite possible, however, that polyhydroxy acids are formed from more highly unsaturated acids by taking up two, or more, molecules of water, but it is unlikely that the hydroxyl groups would occupy adjacent positions.

There still remains the possibility of an unsaturated acid undergoing direct oxidation with formation of a lower fatty acid without intermediate formation of hydroxy, or ketonic acid:—

$$R \cdot CH = CH \cdot COOH \rightarrow R \cdot COOH$$

The occurrence of this change in the animal body cannot be regarded as definitely proved, although Friedmann's experiments on the fate of furfuracrylic acid tend to support this supposition. On injection of the sodium salt of this acid into dogs, unchanged acid was excreted in combination with glycine (furfuracryluric acid) together with pyromucic acid and traces of acetofuran. No optically active β-hydroxy acid was found, such as was observed in the case of cinnamic acid. The acetofuran was undoubtedly derived from furoylacetic acid by loss of carbon dioxide—a reaction analogous to the formation of acetone from acetoacetic acid. It might be assumed that the oxidation took place as follows:—



Friedmann is, however, unwilling to concede that the detection of acetofuran in the urine indicates the fact that any considerable part of the furfuracrylic acid was oxidized by way of furoylacetic acid, since on administering the latter substance to dogs about 50 per cent of the substance was excreted unchanged, and while an active β-hydroxy acid was apparently formed, no pyromucic acid was detected. Friedmann regards these results as a direct experimental proof that α-β-unsaturated acids may be converted into acids with two fewer carbon atoms, without going through the stage of β-ketonic acids. To the writer this conclusion appears likely, but unproven. It appears somewhat unsafe to assume that the fate of subcutaneously injected furoylacetic acid is the same as that of the same substance produced at low concentration in the actual sphere of oxidative change. Moreover, none of the substances of this group appear to be very readily oxidized in the body, as almost 30 per cent of the furfuracrylic acid was recovered unoxidized, but in combination with glycine, after giving to a dog 10 grms. of the acid in the course of five days.

An excellent example of the derivation of a β-ketonic acid from an α-β-unsaturated acid is furnished by Friedmann's demonstration of

acetoacetic acid formation when salts of crotonic acid are perfused through a surviving liver. In this reaction β-hydroxybutyric acid may be first formed which then undergoes oxidation to acetoacetic acid:—

$$CH_3 \cdot CH{=}CH \cdot COOH \rightarrow CH_3 \cdot CHOH \cdot CH_2 \cdot COOH \rightarrow CH_3 \cdot CO \cdot CH_2 \cdot COOH$$

The fate of the phenyl-derivative of crotonic acid offers some peculiarities. This acid, when administered by mouth to dogs, is converted into phenaceturic acid (Knoop), and the same result was obtained when the sodium salt was given subcutaneously to cats (Dakin). At first sight it would appear as if reduction of the unsaturated acid had occurred as well as oxidation:—

$$C_6H_5 \cdot CH{=}CH \cdot CH_2 \cdot COOH \rightarrow C_6H_5 \cdot CH_2 \cdot COOH$$

It is evident that oxidation did not take place with rupture of the side-chain at the double linkage, as in oxidation *in vitro,* for in this case benzoic (hippuric) acid would have been formed. Neither were phenyl-β-γ-dioxybutyric acid nor phenyl-γ-hydroxybutyric acid (phenylbutyrolactone) formed as intermediate stages in the reaction, for the former yields hippuric acid while the latter is very resistant to oxidation in the body. It appears more likely that the unsaturated phenylisocrotonic acid takes up the elements of water to form phenyl-β-hydroxybutyric acid which then undergoes further oxidation. Phenyl-β-hydroxybutyric acid when administered to animals does yield phenylacetic (phenaceturic acid) just as phenyl-isocrotonic acid does (Dakin):—

$$\underset{\text{(Phenylisocrotonic acid)}}{C_6H_5 \cdot CH{=}CH \cdot CH_2 \cdot COOH} \rightarrow \underset{\text{(Phenyl-}\beta\text{-hydroxybutyric acid)}}{C_6H_5 \cdot CH_2 \cdot CHOH \cdot CH_2 \cdot COOH} \rightarrow \underset{\text{(Phenylacetic ac}}{C_6H_5 \cdot CH_2 \cdot COC}$$

It will be noted that the unsaturated acids, crotonic, cinnamic and phenylisocrotonic acid, all yield the same end-products as the corresponding saturated acids, butyric, β-phenylpropionic and γ-phenylbutyric. This was also found to be the case with three unsaturated acids derived from phenylvaleric acid:—

Phenyl-α-β-pentenic acid	$C_6H_5 \cdot CH_2 \cdot CH_2 \cdot CH : CH \cdot COOH$
Phenyl-β-γ-pentenic acid	$C_6H_5 \cdot CH_2 \cdot CH{=}CH \cdot CH_2 \cdot COOH$
Cinnamylidineacetic acid	$C_6H_5 \cdot CH{=}CH \cdot CH{=}CH \cdot COOH$

In each case it was found that the acids on oxidation in the body gave benzoic (hippuric) acid and in each case evidence was obtained by the detection in the urine of substances such as cinnamoylglycine, phenyl-β-hydroxypropionic acid and acetophenone, that the oxidation was indirect, i.e. that the four terminal carbon atoms of the side-chain were removed in two pairs by successive β-oxidation (Dakin).

In considering the changes which the unsaturated acids undergo, the possibility of a shifting of the position of the double linkage must

not be lost sight of. In a great many cases Fittig and others have shown that this change may be effected *in vitro* by simple treatment with acid, or alkali. The conversion of oleic acid into palmitic and acetic acids on fusion with potash, a reaction involving both oxidation and shifting of the double link, may also be recalled in this connexion.

In general it may be said that unsaturated acids undergoing oxidation in the animal body yield products essentially similar to those derived from the corresponding saturated acids; that they may take up the elements of water to form optically active saturated hydroxy acids which then undergo further oxidation; that they may possibly undergo direct oxidation at the double linkage; but dihydroxy acids such as are formed by unsaturated acids in vitro are not intermediate products of these biochemical oxidations.

III. THE ACIDS WITH BRANCHED CHAINS.

The fatty acids with branched chains readily undergo complete oxidation in the animal body. Although these acids are not as important as those of the normal series from a biochemical point of view, the study of their catabolism has shown the occurrence of several interesting types of reaction.

Of the acids containing the isopropyl group,

$$\begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!>CH-$$

isobutyric, isovaleric and isocaproic have been most carefully studied. The first two of these acids are of special importance on account of their relationship with the protein derivatives, α-amino-isovaleric acid (valine) and leucine, from which they are readily formed on oxidation (p. 65). On oxidation *in vitro* with alkaline permanganate, the tertiary hydrogen atom contained in these acids is replaced by a hydroxyl group, while on further oxidation acetone is readily formed[1] (R. Meyer).

On oxidation in the body an entirely different type of reaction occurs, for of these acids referred to only one, namely isovaleric acid, yields acetone on perfusion through a surviving liver.[2]

Baer and Blum similarly found that the administration of salts of isobutyric acid to diabetics was not followed by an increased excretion

[1] On oxidation with nitric acid a similar change takes place, but in addition one of the methyl groups is oxidized to a carboxyl group. Thus, isovaleric acid gives methyl-hydroxysuccinic acid (methyl malic acid) (J. Bredt):—

$$CH_3\cdot\overset{\overset{\displaystyle CH_3}{|}}{C}H\cdot CH_2\cdot COOH \longrightarrow CH_3\cdot\overset{\overset{\displaystyle COOH}{|}}{C}(OH)\cdot CH_2\cdot COOH$$

Isobutyric acid and isovaleric acid are readily oxidized in neutral solution with hydrogen peroxide yielding acetone (Dakin).

[2] Blum, however, has recently stated that isobutyric acid when given to young animals gives rise to acetoacetic acid excretion. The mechanism of this reaction is quite obscure. Further experiments are very desirable in view of the possible contamination of the iso-acid with normal butyric acid. α-oxy-isobutyric acid does not yield acetoacetic acid, but is mostly excreted unchanged together with traces of lactic acid.

of acetone bodies, whereas consumption of isovaleric acid was followed not only by increased excretion of acetone and acetoacetic acid, but also of β-hydroxybutyric acid. Embden later showed that the acetone found after perfusion of the liver with salts of isovaleric acid was derived from acetoacetic acid and hence was produced by a set of reactions entirely different from the *in vitro* oxidations previously referred to:—

$$\begin{array}{l} CH_3 \\ | \\ CH_3\cdot CH\cdot CH_2\cdot COOH \rightarrow CH_3\cdot CO\cdot CH_2\cdot COOH \rightleftharpoons CH_3\cdot CHOH\cdot CH_2\cdot COOH \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad \downarrow \\ \qquad\qquad\qquad\qquad\qquad\quad CH_3\cdot CO\cdot CH_3 \end{array}$$

The biochemical oxidation of isovaleric acid involves the removal of one methyl group from the isopropyl radical. A possible clue to the mechanism of this change was furnished by Baer and Blum's observation of the excretion of traces of d-lactic acid following the administration of iso-butyrates to diabetics:—

$$\begin{array}{ccc} \begin{array}{c} CH_3 \\ | \\ CH_3\cdot CH\cdot COOH \\ \text{(Isobutyric acid)} \end{array} & \rightarrow & \begin{array}{c} OH \\ | \\ CH_3\cdot CH\cdot COOH \\ \text{(Lactic acid)} \end{array} \end{array}$$

This reaction involves the substitution of a methyl group by hydroxyl. It is doubtful, however, if this reaction is general for other acids of this group, for Baer and Blum found that α-methylbutyric acid, $CH_3\cdot CH_2\cdot CH\cdot(CH_3)\cdot COOH$, readily gave rise to β-hydroxybutyric acid when given to diabetics, whereas α-hydroxybutyric acid is incapable of this change. It is perhaps more likely that the methyl group is replaced by a hydrogen atom. That this latter change does *not* take place by intermediate oxidation of the methyl group to carboxyl, followed by loss of carbon dioxide, was shown by the failure of ethyl malonic acid,

$$\begin{array}{l} CH_3\cdot CH_2\cdot CH\cdot COOH \\ \qquad\qquad\quad | \\ \qquad\qquad\quad COOH, \end{array}$$

to yield acetone bodies when administered to a diabetic.

A helpful suggestion as to the mechanism of the oxidation of α-methyl substituted fatty acids has been put forward by Raper. It represents an extension of Knoop's rule of β-oxidation and postulates that the carbon atom of the methyl group which in these acids is in the β-position to the carboxyl group is selectively oxidised rather than the β-carbon atoms of the main chain. Instead of a β-ketonic acid being formed, as with normal fatty acids a derivative of the half aldehyde of malonic acid would result:

$$R-\overset{\displaystyle CH_3}{\overset{|}{C}H}-COOH \longrightarrow R\cdot\overset{\displaystyle CHO}{\overset{|}{C}H}\cdot COOH$$

The higher homologues of malonic semi-aldehyde are not well known, but it is very probable judging by analogy with the lower member and with β-ketonic acids that it would be unstable and part with carbon dioxide. This second reaction would result in the production of the normal aldehyde of an acid containing one less carbon atom than the original methylated acid. This aldehyde on further oxidation or by the Cannizzaro reaction would then give the corresponding normal fatty acid:

$$R\cdot\overset{\displaystyle CHO}{\overset{|}{C}H}\cdot COOH \longrightarrow R\cdot CH_2\cdot CHO \longrightarrow R\cdot CH_2\cdot COOH$$

By such a series of reactions isobutyric acid would give rise to propionic acid, α-methylbutyric acid to *n*-butyric acid and α-methylvaleric acid to n-valeric acid and in support of this view is the fact that the fate of these α-methylated acids is exactly that of the corresponding normal acid which would be produced by demethylation. Thus isobutyric acid like propionic acid gives glucose in the diabetic organism, α-methylbutyric acid like butyric acid gives acetone bodies.

Raper brings further strong support to his theory by showing that, on oxidation of ammonium isobutyrate with hydrogen peroxide, propionic aldehyde is among the products while butyric aldehyde was obtained from ammonium α-methylbutyrate.

Ringer, Frankel and Jonas have shown that isocaproic acid like isobutyric acid yield glucose in the diabetic organism and doubtless the former acid yields the latter by β-oxidation in the normal fashion. Isovaleric acid gives no glucose under similar conditions but yields acetone bodies as already stated. The authors just mentioned have advanced a theory of demethylation in which methyl alcohol is supposed to be split off by hydrolysis. Such a reaction is so much at variance with any reaction known to organic chemistry that it need hardly be considered seriously.

Friedmann showed that β-hydroxy-isovaleric acid gave acetoacetic acid when perfused through a surviving liver, so that this substance may be formed in the oxidation of isovaleric acid. Friedmann found further that salts of α-oxy-isovaleric acid $(CH_3)_2\cdot CH\cdot CHOH\cdot COOH$, pyrotartaric acid $CH_3\cdot CH\cdot(COOH)CH_2\cdot COOH$, citraconic $CH_3\cdot C\cdot(COOH)=CH\cdot COOH$, mesaconic $CH_3\cdot C(COOH)=CH\cdot CH_2\cdot COOH$, and citramalic acids $CH_3\cdot C(OH)\cdot(COOH)\cdot CH_2\cdot COOH$ did not yield acetoacetic acid. Dimethylacrylic acid, on the other hand, readily yields

acetoacetic acid on perfusion. It is probable that this unsaturated acid takes up the elements of water to form β-hydroxy-isovaleric acid, which then undergoes further oxidation (cf. crotonic acid, p. 49):—

$$\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>C{=}CH\cdot COOH \rightarrow \begin{matrix}CH_3\\CH_3\end{matrix}\!\!>COH\cdot CH_2\cdot COOH \rightarrow CH_3\cdot CO\cdot CH_2\cdot COOH$$

(Dimethylacrylic acid) (β-hydroxy-isovaleric acid) (Acetoacetic acid)

Baer and Blum have studied the effect of the administration to diabetics of salts of a number of acids with branched chains other than those previously referred to. These results together with Fried-

Substance		Acetoacetic Acid Formation on Liver Perfusion	Formation of "Acetone Bodies" in Diabetic Organism	Extra Glucose in Diabetic Organism
Isobutyric acid	$\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>CH\cdot COOH$	−	−	+
Isovaleric acid	$\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>CH\cdot CH_2\cdot COOH$	+	+	−
Isocaproic acid	$\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>CH\cdot CH_2\cdot CH_2\cdot COOH$	−		+
Methyl-ethyl-acetic acid (α-methylbutyric acid)	$CH_3\cdot CH_2\cdot \underset{\overset{\vert}{CH_3}}{CH}\cdot COOH$		+	
Di-ethyl-acetic acid (α-ethylbutyric acid)	$CH_3\cdot CH_2\cdot \underset{\overset{\vert}{C_2H_5}}{CH}\cdot COOH$	+[1]	+[1]	
Methyl-ethyl-propionic acid (β-ethylbutyric acid)	$CH_3\cdot \underset{\overset{\vert}{C_2H_5}}{CH}\cdot CH_2\cdot COOH$		+	
Methyl-propyl-acetic acid (α-methylvaleric acid)	$CH_3\cdot CH_2\cdot CH_2\cdot \underset{\overset{\vert}{CH_3}}{CH}\cdot COOH$		−	
Ethyl-malonic acid	$CH_3\cdot CH_2\cdot \underset{\overset{\vert}{COOH}}{CH}\cdot COOH$		−	
α-Hydroxy-isovaleric acid	$\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>CH\cdot CHOH\cdot COOH$	−		
β-Hydroxy-isovaleric acid	$\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>C(OH)\cdot CH_2\cdot COOH$	+		
Dimethylacrylic acid	$\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>C{=}CH\cdot COOH$	+		

[1] Blum and Koppel have identified the ketone derived from di-ethyl-acetic acid as methyl-propyl-ketone. The ketone is derived apparently from the β-ketonic acid $CH_3\cdot CO\cdot CH(C_2H_5)\cdot COOH$ through loss of carbon dioxide.

mann's are collected in the preceding table. It will be seen that only those acids which contain four carbon atoms in a straight chain yield acetoacetic acid. Those with chains of three, or five, carbon atoms do *not* yield acetoacetic acid. On the other hand, not *all* acids with a straight chain of four carbon atoms yield acetoacetic acid, for acids with a carboxyl group in the α-position (ethyl malonic acid), or in the β-position (citramalic acid), evidently undergo oxidation along other lines.

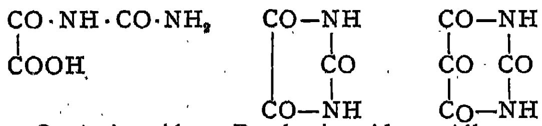
The foregoing results, together with the experiments with β-hydroxy-isovaleric acid and dimethylacrylic acid indicate that within certain natural limitations Knoop's hypothesis of β-oxidation is applicable to the case of fatty acids with branched chains.

In general it may be said that the branched-chain fatty acids, when undergoing oxidation in the body, tend to part with their side-chains and then undergo oxidation along similar lines to those of the corresponding straight-chain normal fatty acids.

IV. THE DIBASIC ACIDS.

The unsubstituted dibasic fatty acids are of relatively little importance in animal metabolism. Only one of them, oxalic acid, is known to be commonly present in the animal organism, but the oxidation in the body of a few of the higher acids has been studied to some extent and the results are of interest in connexion with the general problems of fatty acid oxidation.

Oxalic Acid compared with its homologues and with the monobasic fatty acids is very resistant to oxidation in the animal body. In fact many regard the question whether oxalic acid is oxidized at all as still an open one. A number of observers have declared that oxalic acid is completely unattacked in the animal organism (Gaglio, Wiener, Pohl, Faust and others) while others have found that the oxalic acid excreted in the urine was less than the amount administered (Marfori, Lommel, Autenrieth and Barth, Bakhoven and others). Since, however, the acid was in most cases given by mouth and oxalic acid is susceptible to bacterial decomposition, the results are not convincing and indeed in many cases the analytical methods employed were inadequate. Hildebrandt more recently administered small amounts of oxalic acid subcutaneously to rabbits and found that 60 to 90 per cent was apparently oxidized; and these results were confirmed by Dakin. It must be admitted, however, that the capacity of the body to oxidize oxalic acid, introduced as such, is restricted within very narrow limits. But a number of derivatives of oxalic acid and substances which may under certain circumstances yield oxalic acid in the body are oxidized comparatively easily. Thus, Luzatto and Koehne state that oxaluric acid and parabanic acid, both simple derivatives of oxalic acid,



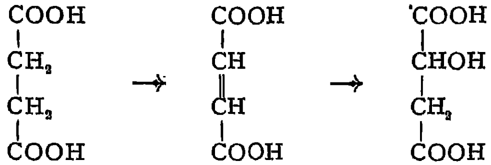
Oxaluric acid   Parabanic acid   Alloxan

are oxidized in the body. Alloxan was also found to be easily oxidized. Later experiments by Lewis throw doubt on the accuracy of these observations so far as parabanic acid is concerned. Glycollic acid ($CH_2OH \cdot COOH$) and glyoxylic acid ($CHO \cdot COOH$), which, when administered subcutaneously in large amounts give rise to a considerable excretion of oxalic acid, may be given in smaller doses, especially by mouth, with the production of little or no oxalic acid excretion. But in the case of the latter acids it is not unlikely that alternative methods of decomposition result in the avoidance of the formation of oxalic acid as an intermediate product. A good account of the known facts concerning the production of oxalic acid in the body is given by Wagrzynowski. Most foodstuffs seem to be potential sources of oxalic acid. Glycerin and gelatin are notably active in this respect.

Malonic Acid, ($COOH \cdot CH_2 \cdot COOH$), in contrast with oxalic acid is readily oxidized in the body. Pohl found that, on giving salts of malonic acid to dogs, there was a barely perceptible rise in oxalic acid excretion and only minute traces of the unchanged acid appeared in the urine. The mechanism of the oxidation of the acid has not been made clear. Ringer, Frankel and Jonas incline to the view that malonic acid may yield glucose in the diabetic animal, but the results do not seem convincing. Tartronic acid, $COOH \cdot CHOH \cdot COOH$, and mesoxalic acid, $COOH \cdot C(OH)_2 \cdot COOH$ were found by Pohl to undergo oxidation in the animal body readily. No intermediate products were detected. Aminomalonic acid is notably toxic.

Succinic Acid, ($COOH \cdot CH_2 \cdot CH_2 \cdot COOH$), and malic acid, ($COOH \cdot CHOH \cdot CH_2 \cdot COOH$), were found by Pohl to be easily oxidized in the body, and their administration led to the excretion of no intermediate products of oxidation. Later experiments have been made by Ohta and by Wise.

Battelli and Stern made the interesting observation that most tissues of the higher animals possess the property of oxidizing salts of succinic acid. They regarded inactive malic acid as the first product of oxidation and this result, if correct, would be a most interesting example of the β-oxidation of a carboxylic acid. The result is brought about by a catalyst which they name 'succinoxydon'. More recent experiments by Thunberg and especially by Einbeck show almost conclusively that the first product of the reaction is the unsaturated fumaric acid, which then in part takes up water with formation of malic acid:—



Succinic Acid Fumaric Acid Malic Acid

The direct production of fumaric acid from succinic acid presents a clear case of dehydrogenation in the sense used by Wieland, and Einbeck was able to trace a correspondence between the amount of oxygen absorbed and the amount of succinic acid oxidised. The final products of the reaction comprise about seventy per cent malic acid and thirty per cent fumaric acid and on digesting salts of fumaric acid with liver tissue its conversion into malic acid takes place to approximately the same extent.[1]

The occurrence of both succinic and fumaric acid in fresh meat has been determined by Einbeck and hence it is reasonable to conclude that malic acid must also occur in the body and indeed large amounts are stated to occur in sheep sweat. This may prove to be of considerable significance, for malic acid may also originate from β-hydroxyglutamic acid and both acids have been shown to yield glucose in the diabetic organism. It has been suggested that this probably occurs through the conversion of malic into lactic acid by loss of carbon dioxide, a reaction known to occur in vegetable organisms. The conversion of lactic acid into glucose in the diabetic organism is of course well established. The actual conversion of malic into lactic acid in the animal body is still hypothetical.

The tartaric acids (i.e. dihydroxysuccinic acids, COOH·CHOH·CHOH·COOH) are less readily oxidized in the body than either malic, or succinic, acid. According to the earlier experiments of Brion the laevo acid is oxidized more readily than the dextro acid, while racemic acid is the most stable. Mesotartaric acid is oxidized to about the same extent as the laevo-tartaric acid. In the light of these results it was somewhat remarkable that Brion found no selective resolution of the racemic acid on administering it to animals; the acid excreted showed no marked optical activity. More recent experiments by Neuberg and Saneyoshi have shown that as a matter

[1] The writer has recently shown that the malic acid derived from succinic or fumaric acid by the action of muscle enzymes is not the optically inactive form as stated by Einbeck and by Battelli and Stern, but is exclusively the laevo variety. The stereochemical similarity of l-malic and d-lactic acid may be significant.

of fact there appears to be no marked difference between the dextro- and laevo-tartaric acids as regards their relative rate of oxidation in the animal body. *d-l*-tartaric acid, therefore, must no longer be classed with the substances which undergo asymmetric decomposition in the animal body.

Glutaric Acid, ($COOH \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH$), undergoes complete oxidation in the animal organism (Marfori). Special interest was directed to this substance together with its higher homologues adipic, pimelic and suberic acids on account of their effect in inhibiting phlorhizin glycosuria in dogs. Later investigations have shown that this effect is mainly concerned with the production of a severe form of nephritis in which both nitrogen and glucose excretion is repressed. No specific effect on glucose metabolism can be ascribed to these acids. Sebacic acid, the next higher homologue of suberic acid was found to produce no marked effect on phlorhizin glycosuria. Nothing definite is known as to the course of oxidation of these higher acids but reference may be made to an experiment by Bodtker on the oxidation of sebacic acid with nitric acid. In addition to succinic, glutaric and adipic acids a small amount of hydroxybutyrofuronic acid (4 Nonanondiacid) was separated. The formation *in vitro* of ketonic acids by direct oxidation of saturated fatty acids is of interest in connection with possible analogous reactions which may occur in the body. The change may be represented as follows:—

$$COOH \cdot (CH_2)_7 \cdot COOH \rightarrow COOH \cdot (CH_2)_2 \cdot CO \cdot (CH_2)_4 \cdot COOH$$

CHAPTER III.

I. THE α-AMINO, α-HYDROXY AND α-KETONIC ACIDS.

THE reasons for classifying togetter the α-amino, hydroxy and ketonic acids are mainly based upon the close structural relationships existing between these substances which frequently make it possible for members of the different groups to undergo mutual interconversion in the animal body. Furthermore, it appears that the processes involved in the oxidation of the three groups of substances have much in common.

The biochemical importance of the substances under consideration hardly requires emphasis including as they do the various amino acids derived from the hydrolysis of proteins,[1] and hydroxy acids such as lactic acid. This significance of the α-ketonic acids has only recently been recognized mainly owing to the admirable researches of Neubauer, and later of Knoop, but it now appears that they play a very prominent rôle in intermediary metabolism.

The amino acids derived from the hydrolysis of proteins are readily oxidized in the animal body, the nitrogen appearing in the urine chiefly in the form of urea, while the carbon is eventually oxidized to carbon dioxide. The earlier experiments of Schultzen and Nencki, and of E. Salkowski, have been amplified by many later workers. While it is true that the animal body eliminates practically the whole of the nitrogen of amino acids, derived from the hydrolysis of protein, in the form of urea and to a lesser extent as ammonia, the ease of this transformation varies considerably with the different amino acids and according to the conditions of the experiment. Thus, according to Stolte's experiments in which various amino acids were injected intravenously into rabbits, glycine and leucine yield urea most completely, while alanine, cystine, aspartic and glutamic acids are less readily metabolized. Phenylalanine and tyrosine injections led to no immediate urea excretion. More recently, Abderhalden and his pupils have made careful studies of the utilization of a number of amino acids and polypeptides

[1] Cf. "Chemical Constitution of the Proteins." This Series. R. H. A. Plimmer.

when fed to dogs. In general it may be said that the decomposition of most amino acids is remarkably complete.[1]

Through the development of new methods of analysis by Folin and Denis and by van Slyke and Meyer much additional information has been gathered concerning the immediate fate of amino acids when absorbed from the intestine or directly introduced into the blood stream. The speed of transference from the blood to the tissues is remarkable. When an increase in the amino acid content of the blood occurs the muscles can take up in the course of a few minutes seventy five or eighty milligrams of amino acid per hundred grams of muscle. Twice this amount may be taken up by the liver but in this case a rapid fall quickly takes place followed by an increase in the urea of the blood. There can be little doubt therefore that the liver plays an important part in the general metabolism of amino acids just as in the case of the fatty acids. The urea formed from amino acids is generally conceded as coming from ammonia split off from the amino acids. The ammonia combines with carbon dioxide and the ammonium carbonate or carbamate is then converted into urea in accordance with Schroeder's well-known observation. Folin and Denis have shown however that much of the ammonia of the portal blood is of bacterial origin due to decomposition in the intestine. Fiske and Sumner have questioned the obligatory formation of ammonia in the catabolism of amino acids and consider that the liver is not the chief site of urea formation from amino acids but their results have failed confirmation by Jansen and are at present not generally accepted.

The production of ammonia and carbon dioxide from amino acids, the first step in the formation of urea, is believed to take place by a process of oxidation in the α-position with formation of an acid with one fewer carbon atom:—

[1] Small amounts of amino acids appear to be constantly present in normal urine—among these glycine has been definitely established (Embden and Marx).

When large amounts of optically inactive amino acids are given to animals the amino acid excreted usually contains an excess of the active component which does *not* occur naturally in the proteins, i.e. the natural component undergoes oxidation more readily, or is more rapidly assimilated (Wohlgemuth).

Occasionally uramido acids, or their anhydrides, the hydantoins, are formed in the urine of animals which have received amino acids, but in some cases these substances are formed after the urine has been passed by the interaction of amino acids and urea (Lippich, Dakin).

$$R \cdot CH_2 \cdot CHNH_2 \cdot COOH + O_2 = R \cdot CH_2 \cdot COOH + CO_2 + NH_3$$

Many examples of this type of change are known to occur in the animal body. Thus the conversion of tyrosine into homogentisic acid in cases of alcaptonuria may be cited, also the formation of ortho- and meta-hydroxyphenylacetic acid from ortho- and meta-tyrosine (Blum, p. 91).

Embden upon applying his method of liver perfusion to the study of amino acid catabolism found that in general the behaviour of the aliphatic α-amino acids was similar to that of the derived fatty acid containing one less carbon atom. Thus leucine gave acetoacetic acid just as isovaleric acid does; valine (α-amino-isovaleric acid) on the other hand does not, neither does isobutyric acid. Oxidation has evidently been effected in the α-position:—

$$\underset{\text{Leucine}}{\begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!>CH \cdot CH_2 \cdot CHNH_2 \cdot COOH} + O_2 = \underset{\text{Isovaleric acid}}{\begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!>CH \cdot CH_2 \cdot COOH} + NH_3 + CO_2$$

A similar reaction may be brought about by bacteria, or yeasts. Thus, Nencki obtained isovaleric acid by the putrefactive decomposition of leucine, and many similar examples are known (p. 103). Moreover, this change can readily be effected *in vitro*. Thus, hydrogen peroxide oxidizes most α-amino acids so as to yield lower fatty acids, ammonia and carbon dioxide (Dakin, Neuberg). In this reaction aldehydes are first formed and they may subsequently undergo further oxidation with formation of acids.[1] Alanine, for example, yields acetaldehyde, acetic acid, ammonia and carbon dioxide, while leucine yields isovaleric aldehyde and isovaleric acid:—

$$CH_3 \cdot CHNH_2 \cdot COOH + O = CH_3 \cdot CHO + NH_3 + CO_2$$

The formation of aldehydes as intermediate steps in the oxidation of α-amino acids has apparently no exact counterpart in animal oxidation, although it appears to occur in certain other biochemical decompositions (cf. p. 101). Instead of an aldehyde being formed in the animal oxidation of α-amino acids to lower acids, it appears that α-ketonic acids are intermediate products. The first evidence for the belief in the intermediate formation of ketonic acids in the oxidation

[1] The amino acids also yield aldehydes on oxidation with alloxan (Strecker, Hurtley and Wooton), with lead peroxide and sulphuric acid (Liebig), with sodium hypochlorite (Langheld), and on exposure of their solutions to light in the presence of iron salts (Neuberg). Amino acids may also be oxidized to cyanides, but the reaction is as yet devoid of biochemical significance.

of α-amino acids was furnished by Neubauer's investigations upon the fate in the body of phenyl-α-aminoacetic acid[1] (p. 68).

That the α-ketonic acids formed by the oxidation of α-amino acids may be further oxidized in the body with formation of a lower acid and carbon dioxide has been frequently observed. Thus, Neubauer found that the α-ketonic acid corresponding to the amino acid, tyrosine, when given to alcaptonurics, is converted into homogentisic acid:—

$$\underset{\text{(Hydroxyphenylpyruvic acid)}}{C_6H_4OH \cdot CH_2 \cdot CO \cdot COOH} \rightarrow \underset{\text{(Homogentisic acid)}}{C_6H_3(OH)_2 \cdot CH_2 \cdot COOH}$$

Similarly, p-chlorphenylpyruvic acid gives p-chlorphenylacetic acid, which is excreted in combination with glycine (Friedmann).[2]

Knoop and Neubauer have suggested that the ketonic acids may be formed from amino acids with intermediate formation of substances of the following type:—

$$R \cdot C \begin{cases} OH \\ COOH \\ NH_2 \end{cases}$$

i.e. hydrates of *imino* acids:—

$$R \cdot C \begin{cases} -COOH \\ =NH \end{cases}$$

These hydrated imino acids which may be regarded as acid derivatives of aldehyde-ammonia compounds are not known in the free state, but undoubtedly would be labile, unstable substances, and on parting with ammonia they would yield ketonic acids.

A possible alternative mode of formation of α-ketonic acids from both α-amino and α-hydroxy acids has been put forward by Dakin and Dudley. It was found that, on digesting dilute solutions of α-amino and α-hydroxy acids with p-nitrophenylhydrazine, small amounts of the bis-nitrophenylhydrazones of the corresponding glyoxals were obtained. Thus, alanine and lactic acid gave the derivative of pyruvic aldehyde (methyl glyoxal). The reaction was found to be general for a wide range of substances. β-amino and β-hydroxy acids gave negative results as might be anticipated. The course of the reaction may be crudely represented as follows:—

[1] It is of interest to note that K. A. H. Mörner found pyruvic acid, $CH_3 \cdot CO \cdot COOH$, and possibly α-ketobutyric acid, $CH_3 \cdot CH_2 \cdot CO \cdot COOH$, among the products of the hydrolysis of various proteins with hydrochloric acid.

[2] The same type of oxidation may be readily brought about *in vitro*. Holleman has shown that the α-ketonic acids are easily oxidized by hydrogen peroxide with formation of carbon dioxide and a lower acid.

$$R\cdot CHOH\cdot COOH \rightleftharpoons R\cdot CO\cdot CHO + H_2O$$
$$R\cdot CHNH_2\cdot COOH \rightleftharpoons R\cdot CO\cdot CHO + NH_3$$

There are various reasons for believing that the above equations only represent a partial truth, but as to the formation of the glyoxals there seems no question, although the reactions are complicated by various side changes. It is also to be remembered that, although the reverse change, namely the formation of hydroxy acids from glyoxals has been observed both inside the body and without, the direct formation of amino acids from glyoxals and ammonia *in vitro* has not been observed. In the body the transformation has been determined, but in this case the synthesis may be via the α-ketonic acid.

However, if the formation of glyoxals, or perhaps more appropriately optically active glyoxal hydrates, are admitted to be formed from α-amino and α-hydroxy acids, it is natural to assume that the α-ketonic acids are derived from them by simple oxidation.

$$\left.\begin{array}{l} R\cdot CHNH_2\cdot COOH \\ R\cdot CHOH\cdot COOH \end{array}\right\rangle R\cdot CO\cdot CHO \rightarrow R\cdot CO\cdot COOH$$

It appears therefore that α-amino acids frequently undergo oxidation in the animal body in such a way that α-ketonic acids are formed, possibly with intermediate formation of a hydrated imino acid or of a glyoxal derivative. The α-ketonic acid in turn may be oxidized with liberation of carbon dioxide and formation of a fatty acid with one fewer carbon atom than the original amino acid. The fatty acid may then undergo complete oxidation with formation of carbon dioxide and water (cf. Chap. II.). The changes may be represented as follows:—

$$R\cdot CH_2\cdot CHNH_2\cdot COOH \rightarrow R\cdot CH_2\cdot CO\cdot COOH$$
$$R\cdot CH_2\cdot CO\cdot COOH \rightarrow R\cdot CH_2\cdot COOH$$
$$R\cdot CH_2\cdot COOH \rightarrow xCO_2 + yH_2O$$

It will appear later that in many cases there are other alternate methods of decomposition for the α-ketonic acids.

Relation of α-Amino to α-Hydroxy Acids.—It was formerly considered probable that the formation of α-hydroxy acids represented the first step in the decomposition of amino acids. This reaction, involving hydrolysis, but not oxidation, is very difficult to imitate directly *in vitro*, except by the use of nitrous acid, and it is doubtful if it occurs in the animal body. But a number of reactions have been observed to take place in the animal body which at first sight appear to be of this type. Thus, Neuberg and Langstein obtained lactic acid from the urine of starving rabbits, when alanine was administered. Embden also obtained lactic acid by perfusing a surviving liver with

alanine. Schotten observed the excretion of mandelic acid, following administration of phenyl-aminoacetic acid, while Blendermann believed that p-hydroxyphenyl-α-lactic acid was present in the urine of rabbits when they were fed large quantities of tyrosine. The constitution of Blendermann's acid is doubtful. Paul Mayer found glyceric acid in the urine of rabbits which had been given α-β-diaminopropionic acid:—

Amino Acid	Hydroxy Acid
Alanine, $CH_3 \cdot CHNH_2 \cdot COOH$	Lactic acid, $CH_3 \cdot CHOH \cdot COOH$
Phenyl-aminoacetic acid, $C_6H_5 \cdot CHNH_2 \cdot COOH$	Mandelic acid, $C_6H_5 \cdot CHOH \cdot COOH$
Tyrosine, $OH \cdot C_6H_4 \cdot CH_2 \cdot CHNH_2 \cdot COOH$	Hydroxyphenyl-α-lactic acid, $OH \cdot C_6H_4 \cdot CH_2 \cdot CHOH \cdot COOH$
α-β-Diaminopropionic acid, $CH_2NH_2 \cdot CHNH_2 \cdot COOH$	Glyceric acid, $CH_2OH \cdot CHOH \cdot COOH$

But many, if not all of these reactions are undoubtedly indirect, i.e. the hydroxy acids are not formed by immediate hydrolysis of the amino acid, but by the reduction of ketonic acids, or in other ways.

Neubauer's observation that both tyrosine and p-hydroxyphenyl-pyruvic acid yield homogentisic acid when given to an alcaptonuric while p-hydroxyphenyl-α-lactic acid does not, also shows that the α-ketonic acids are not readily derived from these hydroxy acids.

Furthermore, Neubauer and Gross found that p-hydroxyphenyl-lactic acid does not readily yield acetoacetic acid, while the corresponding α-amino and α-ketonic acids (tyrosine and p-hydroxyphenyl pyruvic acid) yield acetoacetic acid freely. Similarly, Friedmann found that p-chlorphenylalanine and p-chlorphenylpyruvic acid were converted into p-chlorphenaceturic acid,

$$C_6H_4Cl \cdot CH_2 \cdot CO \cdot NHCH_2 \cdot COOH,$$

on oxidation in the body, while p-chlorphenyl-α-lactic acid did not yield this oxidation product.

It is concluded therefore that the direct conversion of α-amino acids into α-hydroxy acids is not a common biochemical reaction.

The Relationships between α-Amino and α-Ketonic Acids.—O. Neubauer's investigations upon the biochemical oxidation of phenyl-aminoacetic acid, which led to the recognition of the importance of the α-ketonic acids in intermediary metabolism require first consideration.

Schotten found, on administering large amounts of phenyl-amino-acetic acid to dogs, that part of the acid was excreted unchanged, whil

another portion was converted into mandelic acid. Mandelic acid itself is very resistant to further oxidation in the animal body and only yields traces of benzoic acid (hippuric acid) when given to dogs.[1] It appeared at first sight that a simple interchange of (NH_2) for (OH) groups had taken place. On repeating these experiments, Neubauer found that on administering inactive phenyl-aminoacetic acid the following products were excreted in the urine: phenyl-aminoacetic acid containing an excess of the *lævo*-component, *lævo*-mandelic acid, phenylglyoxylic acid and hippuric acid derived from benzoic acid:—

$$\underset{\text{d-l-phenyl-aminoacetic acid}}{C_6H_5\cdot CHNH_2\cdot COOH} \longrightarrow \begin{cases} \text{l-}C_6H_5\cdot CHNH_2\cdot COOH \text{ (l-phenyl-aminoacetic acid)} \\ \text{l-}C_6H_5\cdot CHOH\cdot COOH \text{ (l-mandelic acid)} \\ C_6H_5\cdot CO\cdot COOH \text{ (Phenylglyoxylic acid)} \\ C_6H_5\cdot COOH \text{ (Benzoic acid)} \end{cases}$$

The excretion of lævo-rotatory phenyl-aminoacetic acid following the administration of the inactive acid made it appear probable that the lævo-component was less readily attacked in the organism, and this was definitely proved by administering the lævo-acid which was mainly excreted unchanged together with traces of hippuric acid. There was no appreciable amount of lævo-mandelic acid compared with the previous experiment, although l-phenyl-aminoacetic acid and l-mandelic acid possess a similar stereochemical configuration.

On feeding the *dextro*-phenyl-aminoacetic acid there was found in the urine in addition to phenylglyoxylic acid, *lævo*-mandelic acid, i.e. an apparent optical inversion had occurred. The explanation of this remarkable result was found in the fact that l-mandelic acid was a secondary product formed by the asymmetric *reduction* of phenylglyoxylic acid.[2]

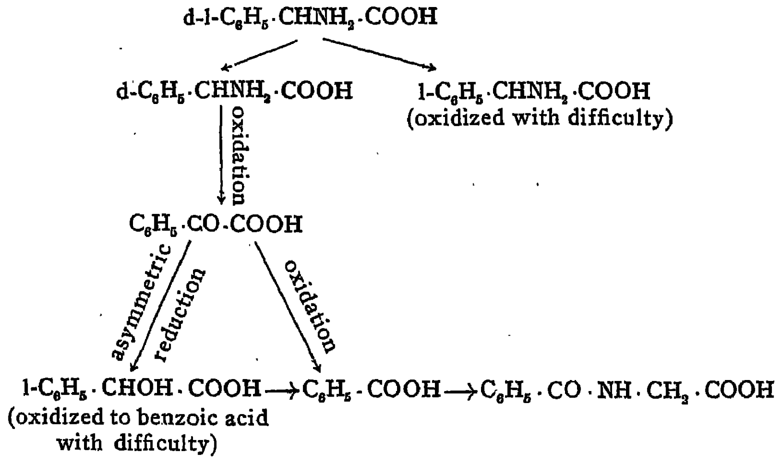
$$C_6H_5\cdot CHNH_2\cdot COOH \xrightarrow{\text{oxidation}} C_6H_5\cdot CO\cdot COOH \xrightarrow{\text{reduction}} C_6H_5\cdot CHOH\cdot COOH$$

Special experiments made with phenylglyoxylic acid confirmed the accuracy of the above conclusion.[3] The oxidation of α-aminophenylacetic acid may be represented as follows:—

[1] Schulzen and Graebe erroneously concluded from their earlier experiments that mandelic acid was readily converted into hippuric acid.

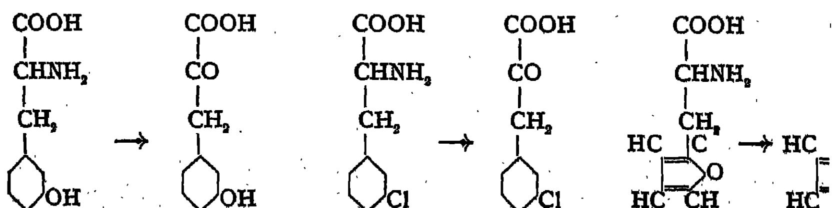
[2] Phenylglyoxylic acid is necessarily optically inactive since it contains no asymmetric carbon atom.

[3] Experiments with p-hydroxyphenyl-aminoacetic acid have shown that this acid undergoes oxidation with formation of the corresponding α-ketonic acid, but no reduction of the ketonic acid to para-hydroxymandelic acid oculd be demonstrated (K. Fromherz).



Neubauer and Fischer by perfusion experiments subsequently showed that the series of changes represented above took place in the liver and might even be demonstrated in minced liver tissue.

Additional examples of the formation of α-ketonic acids from α-amino acids by oxidation in the body have been furnished by Knoop and by Flatow. Knoop observed the excretion of a ketonic acid on administering γ-phenyl-α-aminobutyric acid to dogs and showed that the change was a reversible one. These important results are considered in the following pages. Flatow administered meta-hydroxyphenylalanine (meta-tyrosine), meta-chlorphenylalanine and furylalanine to rabbits; in each case he was able to demonstrate the excretion of ketonic acids. In the case of the first two of these substances the corresponding α-ketonic acid was isolated and definitely identified:—



meta-Hydroxy-phenylalanine | meta-Hydroxy-phenylpyruvic acid | meta-Chlor-phenylalanine | meta-Chlor-phenylpyruvic acid | Furylalanine | Furyl...

Many further experiments with other ketonic acids show that *in general the fate in the body of α-amino and α-ketonic acids is identical, whereas the α-hydroxy acids being presumably secondary*

reduction products may behave differently.[1] *It is therefore assumed that α-ketonic acids are obligate products of the direct oxidation of amino acids, while the hydroxy acids are not directly derived from the amino acids.*

Synthesis of Amino Acids from Ketonic Acids.—Knoop has made a most important study upon the catabolism of γ-phenyl-α-aminobutyric acid showing not only the importance of the α-ketonic acids as oxidation products of the amino acids, but their importance for amino acid synthesis by reduction. A dog received in the course of three days 18 grms. of the inactive amino acid; in the urine the following substances were recovered: *lævo*-phenyl-α-aminobutyric acid, the acetyl derivative of the *dextro*-phenyl-α-aminobutyric acid, *dextro*-phenyl-α-hydroxybutyric acid, hippuric acid and a residue giving the reaction of an α-ketonic acid. The catabolism of the amino acid evidently was similar to that of phenyl-α-aminoacetic acid, and it appeared likely that the hydroxy acid was formed by the asymmetric reduction of the ketonic acid. This supposition was subsequently confirmed by actually administering the sodium salt of the ketonic acid (12 grms.) to a dog and obtaining the dextro-hydroxy acid (2·5 grms.) from the urine. But in addition 0·44 grm. of the acetyl derivative[2] of *dextro*-phenyl-α-aminobutyric acid was obtained, i.e. a synthesis of the amino acid from the ketonic acid, involving reduction, had taken place. The various changes may be represented as follows:—

$$\text{d-l-}C_6H_5\cdot CH_2\cdot CH_2\cdot CHNH_2\cdot COOH$$

$$\swarrow \qquad \searrow$$

$$\text{d-}C_6H_5\cdot CH_2\cdot CH_2\cdot CHNH_2\cdot COOH^{3} \qquad \text{l-}C_6H_5\cdot CH_2\cdot CH_2\cdot CHNH_2\cdot COOH$$

$$\downarrow\uparrow$$

$$C_6H_5\cdot CH_2\cdot CH_2\cdot CO\cdot COOH \rightleftharpoons \text{d-}C_6H_5\cdot CH_2\cdot CH_2\cdot CHOH\cdot COOH$$

$$\downarrow$$

$$C_6H_5\cdot CH_2\cdot CH_2\cdot COOH$$

$$\downarrow$$

$$C_6H_5\cdot COOH \longrightarrow C_6H_5\cdot CO\cdot NH\cdot CH_2\cdot COOH$$

[1] The hydroxy acids in general are less readily oxidized than the corresponding α-ketonic acids. Thus, A. Suwa gave equal amounts (2 grms.) of the sodium salts of p-hydroxyphenyl-α-lactic acid and p-hydroxyphenylpyruvic acid subcutaneously to rabbits. In the former case about 90 per cent of the acid was excreted unchanged, in the latter about 14 per cent of the acid appeared chiefly in the form of p-hydroxyphenylacetic acid. Unchanged ketonic acid was not excreted. When administered to man essentially similar results were obtained, but part of the ketonic acid was asymmetrically reduced to the dextro-hydroxy acid. The differences in ease of oxidation are not nearly so marked in the case of phenyllactic and phenylpyruvic acids.

[2] The mechanism of the acetylation of amino acids is referred to on p. 78.

[3] Excreted in part in form of its acetyl derivative.

Since administration of γ-phenyl-α-hydroxybutyric acid also gav rise to the excretion of a small amount of the dextro-acetyl derivative of γ-phenyl-α-aminobutyric acid, it appears likely that th hydroxy acid may be oxidized in the body to the ketonic aci (a reversible reaction) and so in turn may yield the amino acid The ketonic acid on further oxidation would be expected to yiel phenylpropionic acid. This acid was not isolated from the urin since it readily undergoes β-oxidation yielding benzoic which is excreted as hippuric acid (p. 27). This latter substance was found a previously mentioned.

Knoop's demonstration of the synthesis of α-amino acids fron α-ketonic acids is of fundamental significance for the whole scienc of metabolism.

It was obviously important to determine whether this synthesis o α-amino acids from α-ketonic acids was a general reaction. Utilizin Knoop's suggestion, Embden and Schmitz applied their method o liver perfusion to the problem and found that alanine, phenylalanine and tyrosine, all important protein constituents, might be synthesize in the liver from the ammonium salts of the corresponding ketoni acids:—

$$R\cdot CH_2\cdot CO\cdot COONH_4 \longrightarrow R\cdot CH_2\cdot C\begin{matrix}OH\\ COOH\\ NH_2\end{matrix} \xrightarrow{\text{Reduction}} R\cdot CH_2\cdot CHNH_2\cdot COOH$$

(Ammonium salt of α-ketonic acid) (Imino-acid hydrate) (Amino acid)

The alanine and tyrosine were optically identical with the aminc acids obtained by the hydrolysis of proteins.

Embden, in addition to showing that *d*-alanine might be formec from the ammonium salt of pyruvic acid, when perfused through a surviving liver, showed that ammonium lactate might yield alanin and also that alanine might yield lactic acid. It is evident fron these results that the closest possible relation exists between th amino, hydroxy and ketonic acids.

$$CH_3\cdot CHNH_2\cdot COOH \rightleftharpoons CH_3\cdot CO\cdot COOH \rightleftharpoons CH_3\cdot CHOH\cdot COOH$$

The actual isolation of pyruvic acid from the catabolism of alanine or lactic acid, has not yet been accomplished, but its formation ca hardly be questioned. The conversion of lactic acid into alanine undoubtedly takes place with intermediate formation of pyruvic acid The synthesis of α-aminobutyric acid and nor-leucine from the corresponding ketonic acids has been recorded by Kondo.

A ketonic acid may therefore undergo three types of change (1) It may be oxidized to a lower fatty acid; (2) It may be reduce

to a hydroxy acid; (3) *Its ammonium salt may be reduced to an amino acid.*

(1) $R \cdot CH_2 \cdot CO \cdot COOH + O = R \cdot CH_2 \cdot COOH + CO_2$
(2) $R \cdot CH_2 \cdot CO \cdot COOH + H_2 = R \cdot CH_2 \cdot CHOH \cdot COOH$
(3) $R \cdot CH_2 \cdot CO \cdot COONH_4 + H_2 = R \cdot CH_2 \cdot CHNH_2 \cdot COOH + H_2O$

The conditions determining whether oxidation, or reduction, of an α-ketonic acid shall occur are but slightly understood. The problem is similar to that of acetoacetic acid which also may undergo either reduction or further oxidation (cf. p. 36). In addition, the synthesis of an amino acid by reduction is clearly dependent upon the presence of an adequate supply of ammonia. The presence of much or little glycogen in the liver is also an important factor (Embden and Kraus).

Lactic acid is frequently formed in conditions involving active tissue breakdown combined with insufficient oxidation, such as excessive exercise (Ryffel, Feldman and Hill), or in cases of restricted oxygen supply (Araki). Its origin is readily understood in the light of the foregoing facts. Studies on the fate of the stereoisomeric lactic acids have been made by Parnas. He finds that the dextro acid normally present in animal tissues is non-toxic and almost completely burned when its salts are given subcutaneously to rabbits. The laevo acid is less completely oxidized and in very large doses is somewhat toxic. The inactive acid is asymmetrically attacked in the body so that some of the laevo acid is excreted, but the amount of the *l*-component that is oxidised is greater than when the laevo acid is given alone.

It is of interest to note that all of the three types of reaction which the α-ketonic acids are believed to undergo in the body may be readily imitated *in vitro*. The oxidation of α-ketonic acids to lower acids is readily effected with hydrogen peroxide (Hollemann); their reduction to hydroxy acids may be brought about by sodium amalgam and other reducing agents. The possibility of reducing the ammonium salts of ketonic acids to α-amino acids was shown by Knoop, who obtained phenylalanine by the reduction of ammonium phenylpyruvate with sodium amalgam (cf. also Erlenmeyer and Kunlin, and de Jong).

The Relations between the Carbohydrates and the α-Amino Hydroxy, and Ketonic Acids.—The close relation existing between lactic acid with the carbohydrates on the one hand and with alanine on the other led Knoop to surmise that amino acids might be derived indirectly from the carbohydrates and Embden showed this to be the case. On perfusing a liver rich in glycogen it was found that

alanine was formed, while, when a glycogen-free liver was used, no appreciable amount of alanine was found. The glycogen was undoubtedly converted into lactic acid, which in turn gave alanine with intermediate formation of ammonium pyruvate. The demonstration of this relationship between the amino acids and the carbohydrates is of great importance.

The reverse change to that just referred to, namely, the conversion of certain amino acids and lactic acid into glucose has long been known to occur in the diabetic organism. Lusk and his pupils made the most exact studies upon the conversion of amino acids into glucose by making use of starving dogs rendered fully diabetic by phlorhizin. Depancreatised dogs and human diabetics have also been made use of by others, but the conditions are harder to control and the results less easy of interpretation than when phlorhizin is used. Lusk's experiments showed that glycine, alanine, aspartic and glutamic acids all furnished "extra glucose" in the phlorhizinised dog. Other amino acids were later investigated by other workers including the writer.

In general it may be stated that the biologically important amino acids could be divided into three groups. (1) Those capable of furnishing glucose in the diabetic organism. (2) Those capable of giving acetoacetic acid in the diabetic organism or perfused liver. None of these were capable of producing glucose and conversely none of those in class (1) which gave glucose were also acetoacetic formers. (3) A few amino acids such as lysine and tryptophan which gave neither glucose nor acetoacetic acid. It is an interesting fact that from the standpoint of nutrition the amino acids which are least essential to maintenance or growth will be found in groups 1 and 2 while the indispensable ones will be found in group 3. The explanation of this observation possibly may be found in the ability of the body to supply itself by synthesis with those amino acids whose catabolic paths lead by glucose, lactic acid or acetoacetic acid, all of which are products common to many metabolites. The following table (p. 75) contains most of the available results.

In nearly all cases the experimental results recorded above are unequivocal, but occasionally it is hard to decide that any particular substance yields absolutely no glucose, although it is easy to differentiate between those yielding much and those giving little or none. Ordinarily not much significance should be attached to an "extra glucose" excretion which does not amount to at least twenty per cent of the substance administered. Leucine and histidine in some experiments

Substance	Increased Glucose in Diabetic Animal	Acetoacetic Acid formation in perfused Liver or Diabetic Animal
Glycine	+	−
d- *l*-Alanine	+	
l-Alanine	+	−
d- *l*-Serine	+	
l-Cysteine	+	
l-Aspartic Acid	+	−
d-Glutamic Acid	+	−
β-Hydroxyglutamic Acid	+	
α-Aminoisobutyric Acid		−
d- *l*-Valine	−	−
l-Leucine	−	+
d- *l*-Leucine	− (?)	+
d- *l*-Isoleucine	−	(?)
α-Amino-n-caproic Acid (Norleucine)		−
l-Proline	+	−
d-Ornithine	+	−
d-Lysine	−	−
d-Arginine	+	−
l-Histidine	+ (?)	− (?)
d- *l*-Phenylalanine	−	+
l-Tyrosine	−	+
Glycollic Acid	−	(?)
d- *l*-Lactic Acid	+	−
l-Lactic Acid	+	
d-Lactic Acid	+	−
α-Hydroxyisobutyric Acid		−
d- *l*- α-Hydroxyisobutyric Acid	−	
Malic Acid	+	
Glyoxylic Acid		−
Pyruvic Acid	+	+
Glyoxal	−	

have given small amounts of glucose but the weight of evidence is against leucine as a source of sugar while histidine is doubtful.

Excluding the doubtful cases just referred to and the special case of glycine which will be discussed later, it is found that the yield of glucose obtained from the glucogenetic amino acids always approximates that obtainable from the conversion of three carbon atoms into sugar. Thus alanine with three carbon atoms gives glucose quantitatively, aspartic acid with four carbon atoms gives three fourths of its carbon as glucose, while glutamic and hydroxyglutamic acids, proline and ornithine with five carbon atoms give close to three-fifths of their carbon as glucose. When the known interconvertibility of lactic acid, with three carbon atoms, alanine and glucose are considered together with the preceding facts, it appears an attractive hypothesis to consider lactic acid as an obligate step in the conversion

of all glucogenetic amino acids into glucose. Some of the possible types of change involved in lactic acid formation from the amino acids containing five carbon atoms have been discussed by the writer who is inclined to consider malic acid as a possible intermediate stage. Further experimental evidence is requisite, however, before the scheme deserves serious consideration. Details regarding the mechanism of the lactic acid $\rightleftarrows$ glucose conversion will be found in the chapter dealing with carbohydrates.

The question of the formation of glucose from glycine in the diabetic organism is full of difficulty. There can be no question as to apparent occurrence of the change. An »extra glucose« excretion of over fifteen grams has been observed by Lusk to follow the consumption of twenty grams of glycine, corresponding to an apparent approximately quantitative conversion. But glycollic acid, the corresponding hydroxy acid yields no glucose nor does glyoxal which theoretically might be produced from glycine (Greenwald). Glyoxylic acid, the corresponding keto acid, appears not be formed from or to give glycine in the body. On the other hand, glycollic aldehyde $CH_2OH \cdot CHO$ was shown by Woodyatt and Sansum apparently to give some glucose in diabetic animals but the substance proved to be too toxic for very satisfactory results and they refrain from concluding that a direct glucose synthesis had occurred. Similar experiments by Woodyatt and by Cremer are indecisive. Glycine gives formaldehyde on oxidation and this substance theoretically at least might polymerise to glucose, but even if this happened it would only account for half the observed yield of glucose. There still remains the possibility that the administered glycine present in large amounts may by mass action displace other amino acids from the tissues which then furnish glucose. It is possible that the stimulating effect of glycine on metabolism observed by Lusk may be related to some such change. At present the mechanism of sugar formation from glycine must be considered as completely unsolved.

It appears rather anomalous that valine should give no glucose in the diabetic organism, since it would be expected that its behaviour would be similar to that of isobutyric acid into which it might be converted by oxidation. Isobutyric acid gives glucose freely, but optically inactive valine gave none in Dakin's experiments.

The relation of the biochemically important amino acids to glucose e diabetic organism may be summarised as follows:

(1) The amino acids from proteins which yield glucose freely in the diabetic organism are all those containing two, three, four and five carbon atoms except valine.

(2) Arginine is the only amino acid with more than five carbon atoms which may furnish glucose freely and in this case it evidently comes from ornithine with five carbon atoms into which it is converted by arginase.

(3) All the straight chain amino acids yield sugar except lysine.

(4) The amino acids with branched chaind, including valine, leucine and isoleucine furnish little or no sugar.

(5) Proline is the only cyclic amino acid known to yield much glucose. Hydroxyproline has not yet been investigated. The aromatic amino acids do not yield glucose.

(6) The close structural relations between ornithine, proline, glutamic and β-hydroxyglutamic acids, all of which yield about three fifths of their carbon as glucose, suggests that their catabolic path may be similar and it is not improbable that lactic acid may be an intermediate product.

Lactic and malic acids are the only hydroxy acids which are known to give glucose in the animal body and it is not improbable that malic is converted into lactic as a first step. The fact that both *d*- and *l*-lactic acids, as well as *d*- and *l*-alanines are quantitatively transformed into glucose in the diabetic organism lends support to the hypothesis that some common optically inactive intermediate product, such as pyruvic aldehyde or dihydroxyacetone, is concerned in the process. The discussion of these possibilities is reserved for the section on carbohydrates.

The relation of pyruvic acid to glucose remains to be considered. The early experiments of Paul Mayer showed that when pyruvic acid was given to well nourished normal rabbits hyperglucaemia and glycosuria resulted and lactic acid was found in the urine. Curiously enough he failed to detect glucose formation when the substance was given to diabetic animals. On the other hand Ringer as well as Dakin and Janney had no difficulty in observing the conversion although the results are not nearly so regular as with lactic acid. It appears that only such pyruvic acid as undergoes reduction to lactic acid is converted into glucose. Other forms of decomposition are undoubtedly available and a clue to these has been secured by Embden and Oppenheimer. These authors found that on perfusion through the liver, salts of pyruvic acid gave positive indications of acetoacetic acid formation in seven out of twelve experiments. The yields how-

ever were not large. The formation of acetoacetic acid is ascribed to acetaldehyde formation which, as Friedmann showed, undergoes aldol condensation and oxidation with formation of acetoacetic acid. The relationships of pyruvic acid and its various biochemical transformations may be represented as follows.

$$\begin{tikzcd}
 & & \text{α-Lactic Acid} \arrow[r, shift left] \arrow[dll, shift left] & \text{Glucose} \arrow[l, shift left] \\
\text{Pyruvic Acid} \arrow[urr, shift left] \arrow[r, shift left] \arrow[drr] & \text{Alanine} \arrow[l, shift left] & & \\
 & & \text{Acetaldehyde} \arrow[r] \arrow[dl] \arrow[d, shift left] & \text{Acetoacetic Acid} \\
 & \text{Acetic Acid} & \text{Ethyl Alcohol} \arrow[u, shift left] &
\end{tikzcd}$$

Acetylation of Amino Acids.—It will be recalled that Knoop found that the amino acid synthesized in the body from γ-phenyl-α-ketobutyric acid was excreted in the form of its acetyl derivative :—[1]

$$C_6H_5 \cdot CH_2 \cdot CH_2 \cdot C \begin{cases} H \\ COOH \\ NH \cdot CO \cdot CH_3 \end{cases}$$

(γ-phenyl-acetylaminobutyric acid)

A little later Neubauer and Warburg found that, on perfusing surviving livers with blood containing phenyl-aminoacetic acid, the acetyl derivative of the lævo-amino acid was formed; subsequently the optical antipode, d-phenyl-acetylaminoacetic acid, was found by Neubauer and Fromherz among the products of the action of yeast upon the same amino acid. A similar excretion of an optically active acetyl derivative of an amino acid was observed on feeding inactive p-methyl-phenylalanine to an alcaptonuric subject (Dakin). The formation of these acetyl derivatives appears to be of some significance. Knoop from analogy with a reaction observed by Erlenmeyer and Kunlin and by de Jong suggests that acetylation is effected by means of pyruvic acid. When pyruvic acid is mixed with ammonium carbonate, a rise in temperature followed by loss of carbon dioxide occurs and acetylalanine is formed :—

[1] The possibility of the occurrence of acetylation in the body had already been shown by the fact that certain foreign aromatic substances are excreted as N-acetyl derivatives, e.g. p-nitrobenzaldehyde gives p-acetylaminobenzoic acid. Moreover, Baumann and Preusse's mercapturic acids are acetylated cysteine derivatives :—

$$R \cdot S \cdot CH_2 \cdot CH \cdot NH \cdot COCH_3 \cdot COOH$$

$$
\begin{array}{ccccc}
CH_3\cdot CO\cdot COOH & & CH_3\cdot CH\cdot COOH & & \\
 & & | & & \\
 & +NH_3 \rightarrow & NH & +CO_2+H_2O & \\
 & & | & & \\
CH_3\cdot CO\cdot COOH & & CO\cdot CH_3 & & \\
\text{Pyruvic acid} & & \text{Acetylalanine} & &
\end{array}
$$

This reaction involves a simultaneous oxidation and reduction—one molecule of pyruvic acid is reduced to an amino acid (alanine), while the second yields acetic acid (acetyl group) and carbon dioxide. It is probable that reactions of this type will be found to occur in the animal body for it is becoming increasingly clear that metabolic processes may include many reductions which at first sight might appear unlikely for thermodynamic reasons; the necessary energy is derived from the oxidation of a second molecule of reacting material (cf. "Cannizzaro Reaction," p. 140).

Other Amino Acids.—Brief mention must be made of the oxidation of some other amino acids than those previously referred to :—

Cystine,

$$
\begin{array}{l}
S-CH_2\cdot CHNH_2\cdot COOH \\
| \\
S-CH_2\cdot CHNH_2\cdot COOH
\end{array}
$$

or *cysteine*, $CH_2\cdot SH\cdot CH\cdot NH_2\cdot COOH$, the sulphur containing amino acid derived from proteins, when fed to a normal animal undergoes extensive oxidation. J. Wohlgemuth fed cystine to rabbits and showed that not only the inorganic sulphates of the urine were much increased but thiosulphates were also excreted and the "neutral" or "unoxidized" sulphur of the urine was higher than normal. The sulphur content of the bile was also higher than normal. Blum obtained essentially similar results.

Taurine, $CH_2\cdot NH_2\cdot CH_2\cdot SO_3H$, which is found in the bile combined with cholic acid as taurocholic acid, appears to be derived from cysteine. G. von Bergmann found that on giving cystine to dogs provided with a biliary fistula the taurine of the bile was not increased but by previously administering excess of sodium cholate to the animal, taurine amounting to twice the normal quantity was excreted. Taurine must therefore be regarded as a product of cystine metabolism. E Friedmann had previously shown the possibility of converting cystine into taurine by purely chemical methods. Cystine (cysteine) on oxidation with bromine yields cysteic acid, which on heating with water to 240° loses carbon dioxide with formation of taurine :—

$$
\begin{array}{ccccc}
COOH & & COOH & & \\
| & & | & & \\
CHNH_2 & \rightarrow & CHNH_2 & \rightarrow & CH_2\cdot NH_2 \\
| & & | & & | \\
CH_2(SH) & & CH_2(SO_3H) & & CH_2(SO_3H) \\
\text{(Cysteine)} & & \text{(Cysteic acid)} & & \text{(Taurine)}
\end{array}
$$

There is no evidence to show that the whole of the cysteine supplied to the body is eventually converted into taurine, and indeed such a suggestion appears improbable.[1]

The sulphur of cystine is readily removed by oxidation *in vitro.* Breinl and Baudisch oxidized cystine with hydrogen peroxide and obtained the whole of the sulphur in the form of sulphuric acid. On complete oxidation with alkaline permanganate cysteine gives oxalic, sulphuric, acetic and carbonic acids, ammonia and free sulphur. Pyruvic acid appears to be an intermediate product (Denis.) Cystine may be converted into pyruvic acid by a variety of chemical methods (Baumann, Dewar and Gamgee). This relationship may have biochemical significanc, since cysteine gives glucose like pyruvic acid in the diabetic animal.

An interesting metabolic abnormality is found in the condition known as cystinuria in which the patient excretes considerable quantities of cystine. Apparently cystinurics are capable of oxidizing cystine when administered as such, but for some not yet understood reason, they fail to fully catabolize the cystine arising in the course of ordinary endogenous metabolism.[2]

Serine.—Nothing definite is known of the fate in the animal body of serine, the oxygen analogue of cystine. On exposure of its aqueous solution to sunlight or on oxidation with hydrogen peroxide, serine yields glycollic aldehyde—the simplest sugar (Neuberg).

$$\underset{\text{Serine}}{\begin{array}{l}COOH\\ |\\ CHNH_2\\ |\\ CH_2OH\end{array}} + O \longrightarrow \underset{\text{Glycolaldehyde}}{\begin{array}{l}CHO\\ |\\ CH_2OH\end{array}} + CO_2 + NH_3$$

Paul Meyer is inclined to the belief that glycollic aldehyde undergoes condensation in the body with formation of glucose, since the latter sugar is excreted when large quantities of glycollic aldehyde are given to rabbits. Serine is converted into glucose in the diabetic animal.

Aspartic and Glutamic Acids.—It was previously mentioned that aspartic and glutamic acids when given to diabetic animals lead to the

[1] E. Salkowski found that on administering taurine to rabbits the excretion of sulphates and especially "neutral" sulphur was increased; thiosulphates were also present. These results were not observed when taurine was fed to man or to dogs. See also paper by Schmidt and Allen.

[2] This subject cannot be further discussed here. The reader is referred to Garrod's "Inborn Errors of Metabolism". Oxford Medical Publications, 1909.

excretion of considerable amounts of glucose. Reference to the possible mechanism of change has already been made. As to the mode of catabolism of these acids in the normal organism nothing definite is known.[1]

The Diamino Acids and Histidine.—The mode of catabolism of the diamino acids is obscure. Arginine undoubtedly may undergo hydrolysis by the enzyme arginase yielding urea and ornithine (Kossel and Dakin).

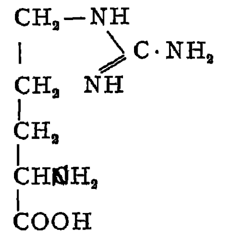
$$\underset{\text{Arginine}}{H_2N \cdot C(=NH) \cdot NH \cdot CH_2 \cdot CH_2 \vdots \cdot CH_2 \cdot CHNH_2 \cdot COOH} + H_2O$$
$$= NH_2 \cdot CO \cdot NH_2 + \underset{\text{Ornithine}}{H_2N \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CHNH_2 \cdot COOH}$$

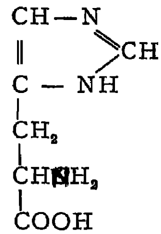
W. H. Thompson has shown that when arginine is administered to a dog an amount of urea corresponding to that liberated by the action of arginase is rapidly excreted in the urine, while an additional quantity presumably derived from the ornithine is more slowly excreted. The intermediate steps in the catabolism of both ornithine and its homologue, lysine, are unknown. Cystinuric patients are apt to excrete tetramethylenediamine and pentamethylenediamine in the urine, and these substances are undoubtedly derived from lysine and ornithine, but it is improbable that these bases represent obligate steps in the normal catabolism of the diamino acids :—

$$\underset{\text{Lysine}}{H_2N \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CHNH_2 \cdot COOH}$$
$$= \underset{\text{Pentamethylenediamine}}{H_2N \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot NH_2} + CO_2$$

A most interesting relationship between arginine and histidine on the one hand and the purine bases on the other has been shown to exist by Ackroyd and Hopkins. When both bases are removed from the food of rats the allantoine excretion in the urine is much decreased, allantoine being the main endproduct of purine metabolism in the rat. It is believed that the allantoine originates to a large extent from histidine and arginine. Since the addition of either base singly to the food prevents the extreme reduction in allantoine excretion, the possibilility of their mutual interconversion is suggested or at least a common metabolic path. As Ackroyd and Hopkins have pointed out the similarity in structure between the two bases is striking when the formulae are compared as follows:

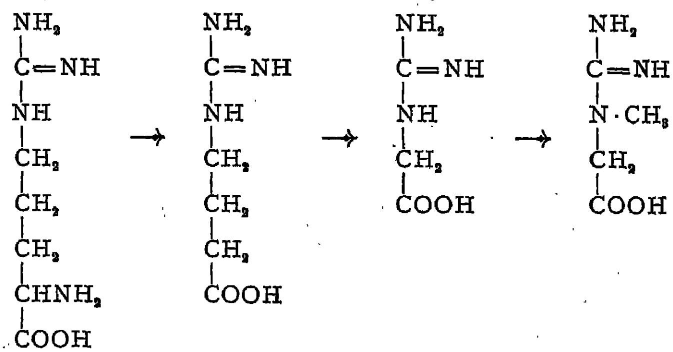
[1] Jastrowitz has shown that a small increase in the oxalic acid in the urine is found after administering large amounts of aspartic and glutamic acids to dogs, but the result is of doubtful significance.


Arginine


Histidine

Abderhalden and Einbeck and Kowalevsky had previusaly failed to observe any formation of allantoine when histidine was given to dogs, but the experimental conditions of their experiments were clearly far less favourable than those of Ackroyd and Hopkins The details of purine and allantoine formation from histidine and arginine are still obscure.

Attention may be drawn to a suggestion as to the possible origin of creatine from arginine. It is possible to oxidize arginine to γ-guanidinebutyric acid *in vitro* with barium permanganate (Kutscher),[1] and such a change might well occur in the living organism. Guanidinebutyric acid on further oxidation in the body would probably yield guanidineacetic acid through β-oxidation. The latter substance is closely related to creatine, its methyl derivative. According to Jaffé and also Dorner, methylation of guanidineacetic acid with formation of creatine may actually take place in the body.


Arginine γ-Guanidinebutyric acid Guanidineacetic acid Creatine

It must be admitted, however, that no satisfactory evidence has yet been adduced of the actual formation in the animal body of creatine from arginine. Both Thompson and Inouye consider that their experiments indicate the occurrence of the change.

[1] On further oxidation guanidine and succinic acid are obtained (Bénech & Kutscher).

A number of other possible precursors of creatine have been sought notably by Riesser, who inclines to regard choline and betaine as sources of creatine. Thomas and Goerne showed that ε-guanido-caproic acid was burned in the body without producing creatine. Careful experiments by Baumann and Hines with sarcosine, betaine, choline, methylguanidine, and cyanamide led in some cases to an apparent increase in creatine, but the experiments were not regarded as decisive. The problem awaits satisfactory solution.

Glycine Synthesis in the Body. Brief reference must be made to the question of glycine synthesis in the body. It has long been known that, on giving large amounts of sodium benzoate to animals, an amount of glycine in combination as hippuric acid was excreted which seemed to be greater than could be accounted for by the ordinary store of preformed glycine in the tissue proteins. The most complete experiment of this kind is due to Abderhalden and Hirsch and it definitely proved that glycine was synthesised in the body. The origin of this glycine is not definitely known. Magnus Levy considered it might be formed from the oxidation of amino acids with longer carbon chains, but the idea could not be substantiated experimentally. Haas showed that aminomalonic acid was toxic and could not function as a precursor of glycine. Neither could glycine be detected on perfusing livers with aspartic or glutamic acids. Sassa similarly failed to demonstrate glycine synthesis from ammonia and glyoxylic acid in the liver or intact animal; a reaction similar to the known formation of alanine from pyruvic acid. More recently Knoop has suggested that glycine is produced in the body by the oxidation of α-amino-β-hydroxy acids. The chief basis for this conception is the fact already observed by Dakin that phenylserine is oxidised in the body to benzoic (hippuric) acid and thus does not behave like the non-hydroxylated phenylalanine. It is therefore concluded that oxidation took place in the β-position:—

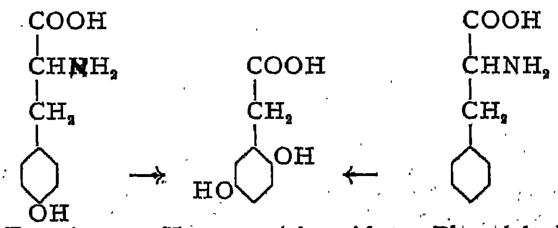
$$C_6H_5\cdot CHOH \mid CHNH_2\cdot COOH \xrightarrow{+O} C_6H_5\cdot COOH + CH_2NH_2\cdot COOH$$

But it must be admitted that while benzoic acid was detected, the formation of glycine is as present hypothetical. The writer is not inclined to regard the experiments with phenylserine as offering more than an interesting speculation as to a possible mode of glycine formation. Should such a reaction be adequately established, the possible production of glycine in the body from serine, β-hydroxy-glutamic acid and possibly hydroxyproline may well be considered.

II. PHENYLALANINE, TYROSINE, TRYPTO-PHAN AND OTHER RELATED AROMATIC SUBSTANCES.

Most aromatic substances do not readily undergo complete oxida-tion in the animal body, the benzene nucleus usually remaining in-tact. The naturally occurring aromatic amino acids, derived from proteins, phenylalanine, tyrosine and tryptophan, however, readily undergo oxidation which involves the disintegration of the benzene ring. The reason for this difference is simply a structural one. It will be seen later that only those aromatic substances possessing a side chain of certain structure undergo oxidation in the animal body with ease.

In considering the nature of the reactions which phenylalanine and tyrosine are likely to undergo in the animal body much attention has been given to the phenomena presented by the curious metabolic ano-maly known as alcaptonuria. In this condition the human organism loses its customary ability to oxidize phenylalanine and tyrosine completely, but instead converts them into homogentisic acid (2·5, di-hydroxyphenylacetic acid). The constitution of this acid was deter-mined by Wolkow and Baumann; they also proved its origin from tyrosine and suspected that phenylalanine might also yield it. Later Falta and Langstein definitely proved the conversion of phenylalanine into homogentisic acid. Tryptophan, on the other hand, when fed to an alcaptonuric does not yield homogentisic acid (Garrod, Neubauer).



Tyrosine ⟶ Homogentisic acid ⟵ Phenylalanine
2·5, Dihydroxyphenylacetic acid

Homogentisic acid is oxidized moderately readily by the normal organism[1] but not by the alcaptonuric. It therefore seemed not impro

[1] H. Embden gave 5·65 grms. homogentisic acid as sodium salt to dog by subcutaneous injection and recovered 1,82 grms. in the urine. O

bable that homogentisic acid was a normal product of tyrosine and phenylalanine catabolism and that the peculiarity of alcaptonuria consisted in failure to carry the oxidation of homogentisic acid to completion. The acceptance of the view that homogentisic acid was a normal intermediary product of phenylalanine and tyrosine catabolism, was more general when Embden, Solomon and Schmidt showed that phenylalanine, tyrosine, and homogentisic acid all gave large amounts of acetoacetic acid when perfused through a surviving liver.[1] It was assumed that tyrosine and phenylalanine were converted into homogentisic acid which in turn gave acetoacetic acid, the latter finally undergoing complete oxidation. There are, however, a number of objections to this assumption, and the writer is of opinion that the path of phenylalanine and tyrosine catabolism does not lie by way of homogentisic acid. The opposite view is, however, very commonly held.

Abderhalden has recently made experiments in which enormous quantities of tyrosine (50 grams) were fed to men but in one case only was a minute amount of homogentisic acid excreted, and he regards this result as insufficient to decide whether homogentisic acid is a main product of normal tyrosine catabolism.

Decomposition of Phenylalanine and Tyrosine in the Normal Organism. Judging by analogy from the experiments of Neubauer and of Knoop upon the phenyl derivatives of α-aminoacetic and α-aminobutyric acids one would expect the first products of the catabolism of phenylalanine and tyrosine to be the corresponding α-ketonic acids, namely phenylpyruvic acid, $C_6H_5 \cdot CH_2 \cdot CO \cdot COOH$ and p-hydroxyphenylpruvic acid, $C_6H_4OH \cdot CH_2 \cdot CO \cdot COOH$. Although the latter acid has not been actually isolated as a metabolic product, there is practically no doubt as to its intermediate formation from tyrosine. Thus like tyrosine it undergoes complete oxidation in the animal body with disruption of the benzene ring; it gives homogentisic acid when fed to an alcaptonuric patient and yields acetoactic acid when perfused through a surviving liver. Moreover the reverse change,

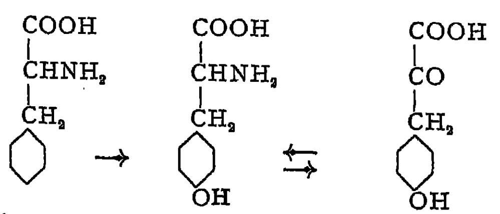
taking 4 grms. of the acid slowly in the course of twenty-four hours he observed no excretion of homogentisic acid in his own case, but on taking 8 grms. more rapidly, over 1 grm. was excreted in the urine.

When homogentisic acid or its salts are given to dogs by the mouth, much of the acid is decomposed by intestinal bacteria with formation of methylhydroquinone (Wolkow and Baumann).

[1] Baer and Blum have shown that when phenylalanine and tyrosine are fed to diabetic patients the excretion of β-hydroxybutyric acid, acetoacetic acid, and acetone may be increased.

namely the synthesis in the liver of natural laevo-tyrosine from *p*-hydroxyphenylpyruvic acid has been observed by Embden and Schmitz. The analogous formation of α-ketonic acids, from the similarly constituted *m*-hydroxyphenylalanine, *m*-chlorphenylalanine and furylalanine has been conclusively shown by Flatow through their detection in the urine after feeding the amino acids to rabbits.

Until recently it was considered almost certain that the first stage in the catabolism of phenylalanine was the formation of phenylpyruvic acid, for the latter acid gave homogentisic acid when fed to an alcaptonuric and can be partially reconverted into *l*-phenylalanine on perfusion through the liver. Moreover it was at first belived that phenylpyruvic acid gave rise to acetoacetic acid in the surviving liver just as phenylalanine does. This latter observation has now been controverted by Embden and Baldes who find that phenylpyruvic acid does not yield acetoacetic acid in the surviving liver but its presence actually inhibits the normal acetoacetic acid formation from other substances. Embden and Baldes therefore believe that phenylpyruvic acid cannot be the first product of phenylalanine catabolism and they believe that phenylalanine is first converted into tyrosine and thence into hydroxyphenylpyruvic acid. In support of this theory they have adduced the strong evidence of the actual isolation of a little tyrosine on perfusing a surviving liver with blood containing added phenylalanine. The first stages in the catabolism of phenylalanine and tyrosine may therefore be represented as follows:



Phenylalanine Tyrosine Hydroxyphenylpyruvic Acid

It will be noted that the evidence on which Embden and Baldes reject the assumption of the formation of phenylpyruvic acid from phenylalanine and insist on its prior oxidation to tyrosine is based on experiments restricted to the liver and it may fairly be questioned whether this holds equally true for phenylalanine catabolism in other organs. Thus the writer has administered phenylalanine intravenously to rabbits so that large amounts were excreted unchanged in the urine without observing the excretion of any tyrosine or other phenolic substance. Moreover, the ready catabolism of para-substituted phenyl-

alanines in which tyrosine formation is either unlikeley or impossible, is against the supposition that phenylalanine is exclusively converted into tyrosine and secondarily into the α-ketonic acid.

It is perhaps worth recalling that phenylpyruvic acid has been found by Bougault and Hemmerlé to occur in two tautomeric forms and it is barely possible that the unsaturated enol form might behave differently from the stable keto-form used by Embden and Baldes for their perfusion experiments.

$$C_6H_5 \cdot CH_2 \cdot CO \cdot COOH \rightleftarrows C_6H_5 \cdot CH = C(OH) \cdot COOH$$

But at least it may be asserted with confidence that phenylalanine may yield tyrosine and that the latter gives p-hydroxyphenylpyruvic acid, while the formation of phenylpyruvic acid from phenylalanine must be regarded as neither proved nor definitely disproved.

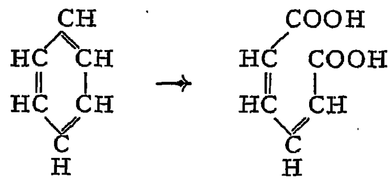
It may be noted that the reduction of phenolic substances such as tyrosine and hydroxyphenylpyruvic acid to unsubstituted benzene derivatives such as phenylalanine has not yet been observed in the animal organism. For the present, therefore, it would appear that the nuclear oxidation of phenylalanine is an irreversible reaction.

Assuming the formation of the α-ketonic acids, what are the next steps in their catabolism? It appears likely that the oxidation of the ketonic acids to the saturated acids with one carbon atom less is not the next step, although this oxidation undoubtedly occurs, but probably at a later stage :—

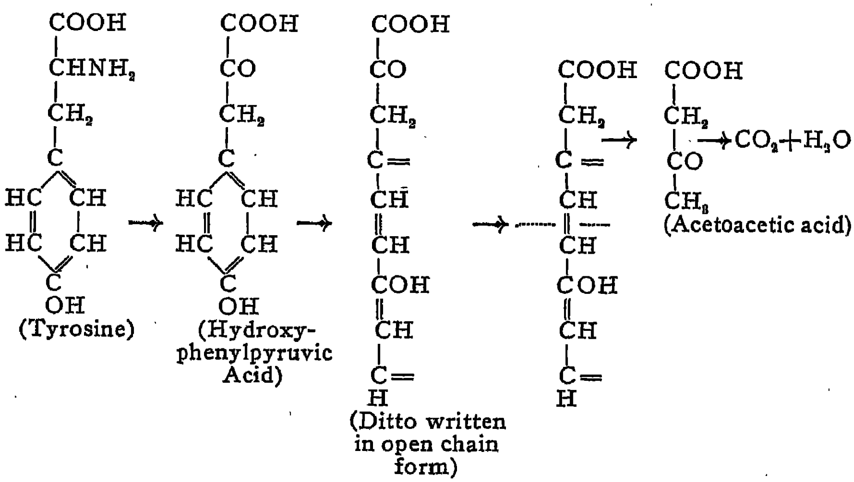
$$R \cdot C_6H_4 \cdot CH_2 \cdot CO \cdot COOH \longrightarrow R \cdot C_6H_4 \cdot CH_2 \cdot COOH$$

The conversion of tyrosine, phenylalanine and their derived α-ketonic acids into homogentisic acid and many other similar reactions are examples of this type of change. But it is found that both phenylacetic acid and p-hydroxyphenylacetic acid, which would be formed by the oxidation of the ketonic acids as suggested, are entirely resistant to further oxidation in the animal body. They do not yield homogentisic acid when administered to an alcaptonuric nor acetoacetic acid when perfused through a surviving liver, but when fed to animals are excreted unoxidized chiefly in combination with glycine (E. & H. Salkowski). They cannot therefore be products of phenylalanine or tyrosine catabolism. We are now confronted with the problem of picturing the conversion of phenylpyruvic acid and p-hydroxyphenylpyruvic acid into acetoacetic acid without prior oxidation of the side-chain. It appears probable that the next change

involves an opening of the benzene ring. An analogy for this somewhat surprising reaction is found in the observation of Jaffé that small quantities of muconic acid could be isolated from the urine of dogs which had been given benzene (p. 136):—[1]



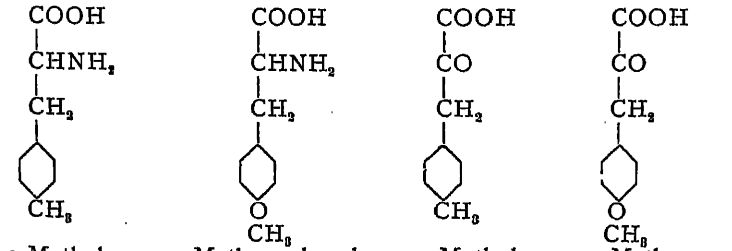
A clear picture of the steps involved in the formation of acetoacetic acid from p-hydroxyphenylpyruvic acid is at present lacking, but Dakin and Wakeman have adduced evidence tending to show that the acetoacetic acid molecule is formed at the expense of two carbon atoms of the nucleus and two of the side-chain. These four carbon atoms are printed in heavy type in the following formulæ:—



The evidence upon which this conclusion is based is largely indirect and can only be reproduced in part. The following points may be mentioned:—

(*a*) Substitution of phenylalanine or tyrosine in the para-position does not necessarily interfere with its complete oxidation in the animal body. Thus p-methylphenylalanine and p-methoxyphenylalanine (tyrosine methyl ether) are readily oxidized when fed to animals.

[1]) Hensel and Riesser state that muconic acid gives acetone or acetoacetic acid on perfusion through the surviving liver.



p-Methyl-phenyl-alanine | p-Methoxyphenyl-alanine (Tyrosine methyl ether) | p-Methyl-phenylpyruvic acid | p-Methoxy-phenylpyruvic acid

(*b*) Para-methylphenylalanine, p-methylphenylpyruvic acid, p-methoxyphenylalanine and p-methoxyphenylpyruvic acid all yield acetoacetic acid when perfused trough a surviving liver.

It may be inferred from these results firstly that phenylalanine derivatives do not necessarily undergo nuclear hydroxylation with formation of tyrosine derivatives; secondly, substances of quinonoid structure which are believed to be necessary precursors of homogentisic acid (p. 92) are not necessarily formed in the catabolism of the aromatic amino and ketonic acids which undergo complete oxidation in the body, since none of the four previously mentioned substances are capable of forming para-quinonoid derivatives.[1]

(*c*) It appears that only those aromatic amino and ketonic acids are capable of yielding acetoacetic acid when perfused through a surviving liver which possess the side-chain—

$$-CH_2\cdot\overset{|}{\underset{|}{C}}\cdot COOH$$

It is essential that the hydrogen of the CH_2 group adjacent to the nucleus be unsubstituted. Thus phenylserine, $C_6H_5\cdot CHOH\cdot CHNH_2\cdot COOH$, although structurally so similar to phenylalanine, is converted into hippuric acid in the animal body (Dakin). A consideration of its formula will show that it cannot yield acetoacetic acid in the same way as phenylalanine and tyrosine do, if the scheme suggested for the catabolism of the latter substance be correct.[2]

[1] Friedmann and Maase fed p-chlorphenylalanine and p-chlorphenylpyruvic acid to dogs, and finding p-chlorphenacetic acid in the urine inferred that the formation of quinonoid substances was necessary for phenylalanine and tyrosine catabolism. This conclusion is open to question since the yield of p-chlorphenylacetic acid did not account for more than 21-36 per cent of the substance fed. Moreover, part of the p-chlorphenylacetic acid may be formed by putrefactive decomposition in the intestine.

[2] Baumann has stated that α-aminocinnamic acid undergoes complete oxidation in the animal body. This statement is, however, incorrect and

(*d*) If the acetoacetic acid be formed from phenylalanine and tyrosine in the manner indicated, an adequate explanation is afforded of the striking differences in the behaviour of other aromatic substances. The reason of the failure of phenylaminoacetic acid, γ-phenyl-α-aminobutyric acid and their derivatives to undergo complete oxidation in the body may reasonably be referred to the structural impossibility of their yielding acetoacetic acid along the lines believed to represent phenylalanine catabolism. Similarly, the phenyl-fatty acids, which when possible undergo β-oxidation, can neither yield acetoacetic acid nor undergo complete oxidation. Furthermore, the failure of tryptophan to yield acetoacetic acid, although it does apparently undergo complete oxidation in the animal body, may be readily explained.

(*e*) With the exception of tryptophan, it appears that practically only those aromatic substances which form acetoacetic acid when perfused through a surviving liver readily undergo complete oxidation in the body. Acetoacetic acid seems commonly to be an obligate stage in the complete oxidation of simple aromatic substances.[1]

Assuming that acetoacetic acid is formed from phenylalanine and tyrosine in the manner suggested, the fate of only five out of the nine carbon atoms in phenylalanine and tyrosine has been accounted for—one present in the carboxyl group appearing as carbon dioxide and four as acetoacetic acid. The disposition of the other four is quite unknown. It is conceivable that another molecule of acetoacetic acid may be formed. The acetoacetic acid formed from the catabolism of phenylalanine and tyrosine would normally undergo further oxidation in the usual fashion, eventually giving carbon dioxide and water (cf. p. 42).

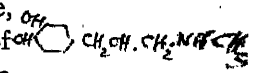
An acid, which was isolated from pathological urines by Schultzen and Riess many years ago, requires mention. The substance was thought to be hydroxymandelic acid and to originate from faulty tyrosine metabolism. Kotake has shown that in reality the substance is laevo-*p*-hydroxyphenyllactic acid. Small amounts of this acid were isolated from urines of animals receiving *p*-hydroxyphenylpyruvic acid, from which it is clearly derived by reduction. On feeding optically inactive hydroxyphenylpyruvic acid, the *l*-com-

apt to lead to confusion. The substance fed by Baumann, prepared by Plöchl's method and believed by him to be α-aminocinnamic acid was found afterwards by Erlenmeyer and by Kunlin to be phenylalanine.

[1] It is of interest to recall the many syntheses *in vitro* of aromatic substances from acetoacetic acid and its derivatives.

ponent was mostly destroyed and the *d* compound excreted. It may be concluded therefore that the *l* acid described by Blenderman was formed by direct asymmetric reduction of hydroxyphenylpyruvic acid and its formation must be regarded as good evidence for the formation of the latter substance in normal tyrosine metabolism.

$$C_6H_4OH\cdot CH_2\cdot CHNH_2\cdot COOH \rightarrow C_6H_4OH\cdot CH_2\cdot CO\cdot COOH$$
$$\rightarrow C_6H_4OH\cdot CH_2\cdot CHOH\cdot COOH.$$

A word must be said as to the possible origin of adrenaline,  3·4 dihydroxyphenylethanolmethylamine, from the aromatic amino acids of proteins. Many attempts to demonstrate adrenaline formation from tyrosine have been made but they appear quite inconclusive. Rosenmund and Dornsaft have recently predicated the intermediate formation of 3·4 dihydroxyphenylserine as a possible parent substance of adrenaline but much more evidence than has yet been produced will be required to give credence to their views.

Further investigation of the detailed mechanism of the catabolism of aromatic amino acids is very desirable.

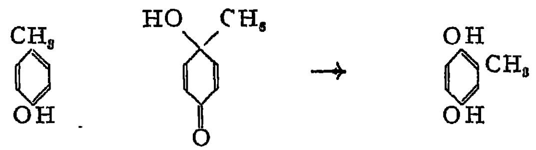
Homogentisic Acid Formation.—The chemical reactions necessary for the conversion of phenylalanine, or tyrosine, into homogentisic acid are rather complex (formulæ, p. 84). The oxidation of the side-chain with loss of one carbon atom is readily intelligible, and, as previously mentioned, there is little doubt that an α-ketonic acid is formed as an intermediate product:—

$$-CH_2\cdot CHNH_2\cdot COOH \rightarrow -CH_2\cdot CO\cdot COOH \rightarrow -CH_2\cdot COOH$$

Homogentisic acid has two hydroxyl groups in positions (2) and (5), while the hydroxyl group in tyrosine is in position (4), and the nucleus of phenylalanine is unsubstituted. Baumann and Wolkow were inclined to assume that the hydroxyl group of tyrosine was reduced by a special micro-organism in the intestine of the alcaptonuric and that subsequently two hydroxyl groups were introduced in positions (2) and (5). This unlikely hypothesis was disapproved by Abderhalden, Bloch and Rona's observation that tyrosine injected subcutaneously in the form of glycyl-tyrosine was converted into homogentisic acid. Blum has shown that of the three *o*, *m*, and *p* hydroxyphenylalanines only the para-compound (tyrosine) yields homogentisic acid, and Neubauer found the same to be true of the three hydroxyphenylpyruvic acids. *The conversion of phenylalanine into homogentisic acid cannot, therefore, take place by direct hydroxylation of the nucleus in position* (2) *and* (5). But Neubauer found that 2·5 dihydroxyphenylpyruvic acid readily gave homogentisic acid when given to an alcaptonuric and hence might be regarded as a probable

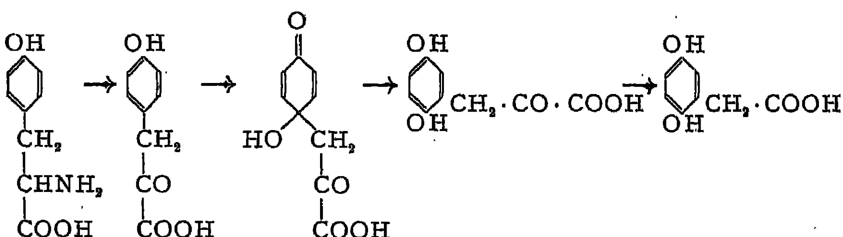
precursor of the latter substance. It was necessary, therefore, to assume that the hydroxylation of the nucleus was effected by some molecular rearrangement involving a shifting of the relative positions of the (OH) group and the side-chain in the tyrosine molecule.

Analogies for this type of change were to be found in the investigations of Bamberger, Kumagai and Wolffenstein, Zincke, Auwers and others upon substances of quinonoid structure, obtained by the oxidation of phenols, which readily undergo intramolecular rearrangement. For example, p-cresol on oxidation with potassium persulphate yields "tolu-quinol" which readily passes over into methylhydroquinone:—



Paracresol Tolu-quinol Methylhydroquinone

It was suggested by E. Meyer, and later by Friedmann and by Neubauer, that a reaction of this type was probably concerned with homogentisic acid formation. Neubauer has put forward the view that tyrosine (I) is converted first of all into hydroxyphenylpyruvic acid, (II) which on oxidation gives a substance of quinonoid structure, (III); the latter body then undergoes intramolecular rearrangement with formation of 2·5 dihydroxyphenylpyruvic acid, (IV). The oxidation of the latter substance to homogentisic acid, (V) is readily understood.



I II III IV V

Phenylalanine is pictured by Neubauer as undergoing a similar set of changes, either by being converted into tyrosine by direct hydroxylation of the nucleus or into phenylpyruvic acid, which on further oxidation gives p-hydroxyphenylpyruvic acid. The latter substance is then converted into homogentisic acid as in the case of tyrosine oxidation.

Neubauer's theory of homogentisic acid formation accords with all the known facts. Each of the substances assumed to be intermediate

products (with the exception of the substance of quinonoid structure which has not been isolated and which undoubtedly would be very unstable) has been shown to yield homogentisic acid when given to an alcaptonuric patient. It must be admitted, however, that chemical analogy for the "wandering" of the $CH_2 \cdot CO \cdot COOH$ group is lacking.[1]

On administering to alcaptonurics para-methylphenylalanine and para-methoxyphenylalanine, i. e. substances incapable of forming quinonoid derivatives, it was found that they did not cause the excretion of any homogentisic acid derivative, but underwent complete oxidation relatively easily (Dakin). Similarly Fromherz and Hermanns have shown that neither meta nor para-tolylalanine, nor *m*-methyltyrosine are capable of producing homogentisic acid. These results may be taken as evidence in favour of the view that a quinonoid substance is a necessary precursor of homogentisic acid, and furthermore that alcaptonurics have not lost the power to effect complete oxidation of phenylalanine and tyrosine derivatives provided their structure is such that homogentisic acid formation is prevented. Alcaptonuria, according to this view, represents a condition in which there is not only an abnormal formation of homogentisic acid but also an abnormal failure to catabolize it when formed.[2] Neubauer and many others, including Garrod, on the other hand, incline to believe that alcaptonuria represents a failure to deal with a normal product of intermediary metabolism, i.e. homogentisic acid.

A number of aromatic substances in addition to those previously referred to have been examined with regard to their ability to form homogentisic acid when given to an alcaptonuric and also as to their fate in the normal organism and their ability to yield acetoacetic acid when perfused through a surviving liver. Many of these observations are of considerable value and are collected in the following table :—

[1] Although p-cresol on oxidation with potassium persulphate gives methylhydroquinone, the methyl group changing its position, the writer was unable to observe the analogous formation of homogentisic acid on oxidizing p-hydroxyphenylacetic acid (unpublished experiments).

[2] Neubauer and Falta have made some comparative observations upon the fate of gentisic acid (2·5 dihydroxybenzoic acid), 2·4 dihydroxybenzoic acid, 3·4 dihydroxybenzoic acid and caffeic acid, 3·4 dihydroxycinnamic acid, in the normal and alcaptonuric organism. The results appeared to show a somewhat diminished capacity of the alcaptonuric for the oxidation of the two first of these substances.

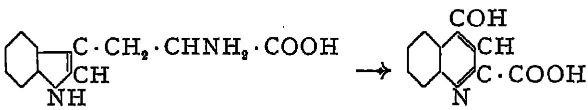
Substance.	Fate in the Normal Organism.[1]	Homogentisic Acid Formation when given to Alcaptonuric.	Acetoacetic Acid Formation when Perfused through Surviving Liver.
Phenylethylalcohol, $C_6H_5 \cdot CH_2 \cdot CH_2OH$	$C_6H_5 \cdot CH_2 \cdot COOH$	—	
Phenylacetaldehyde, $C_6H_5 \cdot CH_2 \cdot CHO$	$C_6H_5 \cdot CH_2 \cdot COOH$		
Phenylacetic acid, $C_6H_5 \cdot CH_2 \cdot COOH$	not oxidized	—	
o- m- & *p*-Hydroxyphenylacetic acids, $C_6H_4(OH) \cdot CH_2 \cdot COOH$	" "		
Homogentisic acid, $C_6H_3(OH)_2 \cdot CH_2 \cdot COOH$	Complete oxidation	+	+
Phenylaminoacetic acid, $C_6H_5 \cdot CHNH_2 \cdot COOH$	$C_6H_5 \cdot COOH$	—	—
Phenylproprionic acid, $C_6H_5 \cdot CH_2 \cdot CH_2 \cdot COOH$	$C_6H_5 \cdot COOH$	—	—
Cinnamic acid, $C_6H_5 \cdot CH{=}CH \cdot COOH$	$C_6H_5 \cdot COOH$	—	
Phenylalanine, $C_6H_5 \cdot CH_2 \cdot CHNH_2 \cdot COOH$	Complete oxidation	+	+
Tyrosine, $C_6H_4OH \cdot CH_2 \cdot CHNH_2 \cdot COOH$	" "	+	+
3·5 Diiodotyrosine, $C_6H_2I_2(OH) \cdot CH_2 \cdot CHNH_2 \cdot COOH$	" "		—
p-Methylphenylalanine, $C_6H_4(CH_3)CH_2 \cdot CHNH_2 \cdot COOH$	" "	Complete oxidation	+
p-Methoxyphenylalanine, $C_6H_4 \cdot (OCH_3) \cdot CH_2 \cdot CHNH_2 \cdot COOH$	" "	Complete oxidation	+
Phenyl-*β*-alanine, $C_6H_5 \cdot CHNH_2 \cdot CH_2 \cdot COOH$	Not oxidized readily		
Phenylserine, $C_6H_5 \cdot CHOH \cdot CHNH_2 \cdot COOH$	$C_6H_5 \cdot COOH$		
Phenyl-*α*-lactic acid, $C_6H_5 \cdot CH_2 \cdot CHOH \cdot COOH$	Complete oxidation	+	+
p-Hydroxyphenyl-*α*-lactic acid, $C_6H_4(OH) \cdot CH_2 \cdot CHOH \cdot COOH$	Oxidation difficult but probably complete[2]	—	Doubtful
o- & *m*-Hydroxyphenyl-*α*-lactic acid, $C_6H_4(OH) \cdot CH_2 \cdot CHOH \cdot COOH$	Oxidation difficult but probably complete	—	
Phenylglyceric acid, $C_6H_5 \cdot CHOH \cdot CHOH \cdot COOH$	$C_6H_5 \cdot COOH$	—	
2·5 Dihydroxyphenyl-*α*-lactic acid, $C_6H_3(OH)_2 \cdot CH_2 \cdot CHOH \cdot COOH$	Complete oxidation	+	
Phenyl-*β*-hydroxypropionic acid, $C_6H_5 \cdot CHOH \cdot CH_2 \cdot COOH$	$C_6H_5 \cdot COOH$		
o-m- & *p*-Hydroxyphenylpropionic acids, $C_6H_4OH \cdot CH_2 \cdot CH_2 \cdot COOH$	$C_6H_4(OH) \cdot COOH$		
Phenylpyruvic acid, $C_6H_5 \cdot CH_2 \cdot CO \cdot COOH$	Complete oxidation	+	—
p-Hydroxyphenylpyruvic acid, $C_6H_4(OH) \cdot CH_2 \cdot CO \cdot COOH$	" "	+	+
o- & *m*-Hydroxyphenylpyruvic acid, $C_6H_4(OH) \cdot CH_2 \cdot CO \cdot COOH$	" "	—	
p-Methylphenylpyruvic acid, $C_6H_4(CH_3) \cdot CH_2 \cdot CO \cdot COOH$	" "		+
p-Methoxyphenylpyruvic acid, $C_6H_4 \cdot (OCH_3) \cdot CH_2 \cdot CO \cdot COOH$	" "		+
2·5 Dihydroxyphenylpyruvic acid, $C_6H_3(OH)_2 \cdot CH_2 \cdot CO \cdot COOH$	" "	+	

[1] End-products of oxidation are alone considered. Substances excreted in combination with glycine, etc., are recorded in the Table in the uncombined form.

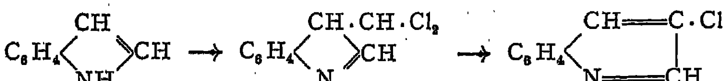
[2] i.e. The aromatic nucleus of that part of the acid which undergoes oxidation is destroyed. No benzoic acid is formed.

Oxidation of Tryptophan. — Tryptophan, i.e. indole-α-aminopropionic acid, undergoes metabolism in the animal body along quite different lines from those followed by tyrosine and phenylalanine. In the human organism it apparently undergoes complete oxidation. No indole derivatives are found in the urine except such as are due to putrefactive decomposition of the amino acid in the intestine, prior to absorption. Neither does tryptophan yield homogentisic acid when given to an alcaptonuric (Garrod, Neubauer) nor give acetoacetic acid when perfused through a surviving liver. The mechanism of the changes involved in the complete oxidation of tryptophan is completely unknown.

When tryptophan is fed to dogs Ellinger noted an increase in the normal excretion of kynurenic acid and was also able to induce the formation of the same substance in rabbits. Kynurenic acid has been shown by Miss Homer to be γ-hydroxyquinoline-α-carboxylic acid and is not identical with the β-carboxylic acid synthesised by Camps as was formerly belived to be the case.



The conversion of tryptophan into kynurenic acid requires the entrance of an additional carbon atom into the indole ring with formation of a quinoline nucleus. This remarkable type of reaction has often been observed *in vitro*. Thus Ellinger obtained β-chlorquinoline together with indole aldehyde by the action of chloroform and potash upon indole (Tiemann-Reimer reaction) and similar results were obtained by Ciamician and co-workers with alkyl indole derivatives[1] (Magnanini).

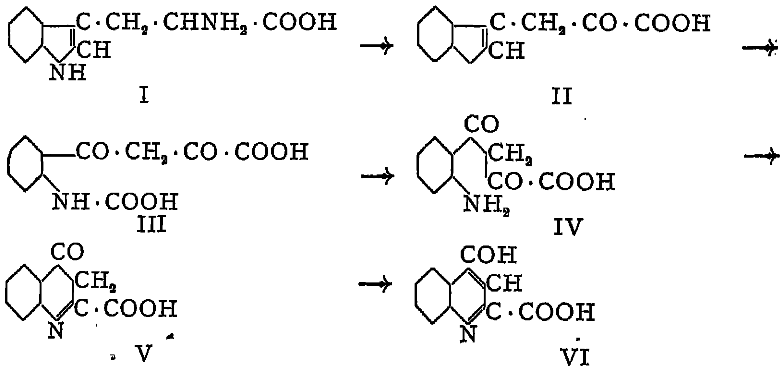


Indole $\qquad\qquad\qquad\qquad$ β-Chlorquinoline

It is possible to picture the conversion of tryptophan into kynurenic acid in many different ways. The following scheme which is that adopted by Ellinger and Matsuoka cannot be regarded as definitely settled but is at least an approximation capable of further

[1] Abderhalden and Kempe have described a by-product obtained in the preparation of tryptophan which they consider to be a hydroxytryptophan of unknown structure. On evaporation with hydrochloric acid, followed by heating, the odour of quinoline was observed. The reaction requires further study.

experimental investigation. Judging by analogy with other amino acids it is natural to suppose that indolepyruvic acid is the first product of tryptophan catabolism and it has been shown that this acid on intravenous administration to rabbits gives considerable accounts of kynurenic acid although rather less than an equivalent amount of tryptophan. The next step is believed to involve the opening of the pyrrole ring with the formation of an acid (III) which parts with carbon dioxide to give aminobenzoylpyruvic acid (IV). Separate experiments with this acid have not yet been made but the conversion of aminobenzoylpyruvic acid into kynurenic acid (VI) with ring closure appears reasonably probable. The changes may be represented as follows:



It was thought that light might be thrown on the accuracy of the above presentation by the examination of the fate of substituted tryptophans. Barger and Ewins as well as Ellinger and Matsuoka have prepared α-methyltryptophan, but on feeding to rabbits no excretion of kynurenic acid nor derivatives of it, was observed. A result of some negative value follows from the observation that α-quinoline-carboxylic acid does not yield kynurenic acid in the rabbit, hence it is safe to assume that kynurenic acid does not result from a direct hydroxylation of a quinoline derivative by a reaction comparable to the formation of phenolic substances from benzene derivatives.

It is doubtful if kynurenic acid is produced in the intermediary metabolism of those animals which do not normally excrete it. When given by mouth to man little or none is excreted in the urine, but if the acid be given subcutaneously it may be excreted in considerable amount. When given to rabbits and dogs by the mouth only part reappears in the urine. Generally speaking kynurenic acid appears to

undergo oxidation in the body with some difficulty (Solomin, Hauser, A. Schmidt).

Nothing definite is known of any intermediate products of normal tryptophan catabolism other than kynurenic acid. This is the more regretable in view of the complete dependance of the animal organism on tryptophan for maintenance and growth. A close relationship undoubtedly exists between tryptophan and the iodine containing active principle of the thyroid isolated by Kendall. Further investigation of the normal metabolism of tryptophan is highly desirable.

Eppinger has recently made an interesting observation in a case of melanuria upon the dependence of pigment formation on tryptophan. He also succeeded in isolating a substance from the urine which appears to be a pyrrole derivative and which apparently represents an intermediate stage in the formation of pigment from tryptophan.

III. THE OXIDATION AND REDUCTION OF AMINO ACIDS BY MICRO-ORGANISMS.

Since a number of the amino acids formed during normal intestinal digestion may be attacked by intestinal bacteria with the formation of various types of substances[1] which subsequently are absorbed, it seemed proper to include a brief account of the chief types of decomposition of the amino acids by micro-organisms.

Most of the products of the bacterial decomposition of the amino acids were isolated in the first instance from mixtures obtained by allowing proteins to undergo putrefaction. As a more exact knowledge of the structure of the amino acids was acquired, the probable origin of most of these decomposition products became clear and experiments were then made upon the decomposition of the amino acids themselves.

The amino acids derived from proteins furnish bacteria, moulds, yeasts and other vegetable forms with a readily available source of nitrogen. The chemical changes involved depend largely upon the character of the organism, the condition of growth especially with regard to the presence or absence of oxygen and the available sources of nutriment other than the amino acids.

In general, it may be said that anærobic bacteria are prone to *reduce* α-amino acids with formation of saturated fatty acids and liberation of ammonia (I). The formation of phenylpropionic acid from phenylalanine is an example of this type of change:—

$$C_6H_5 \cdot CH_2 \cdot CHNH_2 \cdot COOH + H_2 = C_6H_5 \cdot CH_2CH_2 . COOH + NH_3$$

Aerobic bacteria more frequently *oxidize* the α-amino acid to a fatty acid containing one fewer carbon atom, carbon dioxide and ammonia being set free (II). Thus leucine may be converted into isovaleric acid (Nencki):—

$$\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\!CH \cdot CH_2 \cdot CHNH_2 \cdot COOH + O_2 = \begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\!CH \cdot CH_2 \cdot COOH + NH_3 + CO_2$$

[1] Ackermann and Kutscher have suggested the use of the word "Aporrhegma" as a class designation for the various substances derived from the decomposition of amino acids by physiological processes by both vegetable and animal organisms.

Yeasts, on the other hand, have been shown by F. Ehrlich to convert the amino acids into alcohols, carbon dioxide and ammonia (III). Alanine, for example, yields ethyl alcohol:—

$$CH_3 \cdot CHNH_2 \cdot COOH + H_2O = CH_3 \cdot CH_2OH + NH_3 + CO_2$$

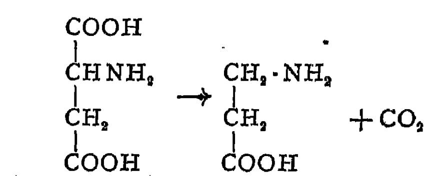
The net result of this last reaction indicates neither reduction nor oxidation but simply hydrolysis and liberation of carbon dioxide. It will be shown, however, that probably both oxidation and reduction are concerned in this change.

I. $R \cdot CH_2 \cdot CHNH_2 \cdot COOH + H_2 \rightarrow R \cdot CH_2 \cdot CH_2 \cdot COOH + NH_3$ (Reduction)
II. $R \cdot CH_2 \cdot CHNH_2 \cdot COOH + O_2 \rightarrow R \cdot CH_2 \cdot COOH + NH_3 + CO_2$ (Oxidation)
III. $R \cdot CH_2 \cdot CHNH_2 \cdot COOH + H_2O \rightarrow R \cdot CH_2 \cdot CH_2 \cdot OH + NH_3 + CO_2$ (Hydrolysis)
IV. $R \cdot CH_2 \cdot CHNH_2 \cdot COOH \rightarrow R \cdot CH_2 \cdot CH_2 \cdot NH_2 + CO_2$ (Amine formation)
V. $R \cdot CH_2 \cdot COOH \rightarrow R \cdot CH_3 + CO_2$ (Carboxylic acid decomposition)

Another type of reaction (IV) very commonly effected by bacteria involves the liberation from the amino acid of carbon dioxide but not ammonia. Amines are formed in this way. Tyrosine, for example, yields p-hydroxyphenylethylamine:—

$$OH \cdot C_6H_4 \cdot CH_2 \cdot CHNH_2 \cdot COOH = OH \cdot C_6H_4 \cdot CH_2CH_2NH_2 + CO_2$$

Details concerning many of the bases thus found, some of which are of great physiological importance will be found in Barger's Monograph on The Simpler Bases. Carbon dioxide is also frequently liberated from the dicarboxylic acids such as aspartic and glutamic acids and from the acids produced by the oxidation of amino acids according to the second reaction (V). The formation of β-alanine and cresol from aspartic acid and tyrosine respectively are examples of this type of change.



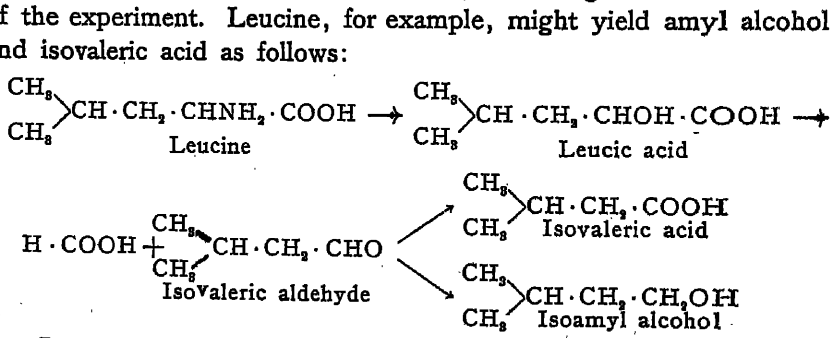
Aspartic acid β-Alanine

A combination of a number of these reactions may be effected by a single organism and different results may often be obtained using the same organism growing under varying conditions. When optically inactive amino acids undergo decomposition by micro-organisms it is usual for both forms to be attacked but at unequal rates. The difference in rate may vary widely so that in some cases a resolution into an active form may be effected. In other cases, e.g. glutamic acid, the two active forms may be decomposed at almost equal rates and no resolution is possible (Neuberg).

Before considering the various decomposition products of the amino acids, reference may be made to the types of change involved in the typical reactions recorded above.

Reduction of the amino acids to saturated acids (I) although easily brought about by anærobic bacteria, is effected *in vitro* with some difficulty. For example, hydriodic acid (sp. gr. 1·96) reduces amino acids when heated in sealed tubes at 220°. Thus glycine gives acetic acid and ammonia. The biochemical oxidation of the amino acids (II) with formation of a fatty acid, ammonia and carbon dioxide is readily imitated *in vitro*, either by the action of hydrogen peroxide, lead peroxide or by exposure of aqueous solutions to sunlight. It is not unlikely that in the biochemical oxidation of the amino acids by bacteria, α-ketonic acids are intermediate products as is the case when they undergo oxidation in the animal body.

The formation of alcohols from amino acids (III) has been shown by F. Ehrlich to be a very general reaction of the amino acids when acted upon by actively fermenting yeast in the presence of much sugar. It is possible to picture this reaction taking place in many different ways (cf. Harden, "Fermentation", this series, p. 81). Ehrlich was inclined to the belief that the amino acid is first converted into the hydroxy acid with liberation of ammonia. The hydroxy acid was supposed then to undergo decomposition into formic acid and an aldehyde, and the latter substance then reduced to the corresponding alcohol or oxidized to the acid, according to the conditions of the experiment. Leucine, for example, might yield amyl alcohol and isovaleric acid as follows:



Recent experiments by O. Neubauer and Fromherz have shown, that amino acids may on the one hand undergo *oxidation* through the action of yeast yielding α-ketonic acids and that the latter substances may then undergo *reduction* with formation of an alcohol and carbon dioxide. For example, they isolated l-phenylaminoacetic acid, l-phenylacetylaminoacetic acid[1], phenylglyoxylic acid, l-mandelic

[1] Cf. Acetylation of amino acids in the animal body, p. 18.

acid and benzyl alcohol from the products of the action of yeast upon d-l-phenylaminoacetic acid. Benzyl alcohol is the final product of the action of yeast upon this amino acid. Neubauer and Fromherz further showed that the phenylglyoxylic acid underwent partial reduction to l-mandelic acid and that the reverse change, namely the oxidation of mandelic acid to phenylglyoxylic acid could also be demonstrated, although this reaction was less vigorous than the former. That the ketonic acids are the probable precursors of the alcohols was also shown by subjecting para-hydroxyphenylpyruvic acid to the action of yeast and obtaining an excellent yield (70 per cent.) of p-hydroxylphenylethyl alcohol (tyrosol). The decomposition of phenylaminoacetic acid may be represented as follows:

$$\text{d-l-}C_6H_5\cdot CHNH_2\cdot COOH$$

$$\swarrow \qquad\qquad \searrow$$

$$\text{d-}C_6H_5\cdot CHNH_2\cdot COOH \qquad\qquad \text{l-}C_6H_5\cdot CHNH_2\cdot COOH$$

$$\downarrow \qquad\qquad\qquad\qquad \downarrow$$

$$C_6H_5\cdot CO\cdot COOH \qquad\qquad \text{l-}C_6H_5\cdot CH(NH\cdot CO\cdot CH_3)\cdot COOH$$

$$\swarrow \qquad \searrow$$

$$\text{l-}C_6H_5\cdot CHOH\cdot COOH \qquad C_6H_5\cdot CHO + CO_2$$

$$\searrow \qquad \swarrow$$

$$C_6H_5\cdot CH_2OH$$

The hydroxy acids do not appear to be obligate steps in the conversion of the ketonic acids into alcohols, since the yield of tyrosol obtained from p-hydroxyphenyl-α-lactic acid was only about 5 per cent. of that obtained from the ketonic acid. It appears far more likely that the ketonic acid undergoes decomposition so as to yield carbon dioxide and an aldehyde, which is then reduced to the alcohol. Moreover, Neuberg and his pupils have described an enzyme in yeast which they name "carboxylase" and which has the property of decomposing α-ketonic acids, such as pyruvic acid and oxalacetic acid, with formation of an aldehyde and carbon dioxide. Benzaldehyde has been shown to be capable of reduction to benzylalcohol by yeast, and has been found by Ehrlich among the products of the action of yeast npon phenyl-α-aminoacetic acid. In this particular case it may, however, have been artificially formed from the phenylglyoxylic acid during analysis. The reduction of aldehydes to alcohols by yeast appears to be a general reaction.

The typical reactions involved in the formation of an alcohol from an α-amino acid appear to be as follows:

$$\underset{(\alpha\text{-Amino acid})}{R\cdot CH_2\cdot CHNH_2\cdot COOH} \xrightarrow{\text{oxidation}} \underset{(\alpha\text{-Ketonic acid})}{R\cdot CH_2\cdot CO\cdot COOH} \rightarrow$$

$$\xrightarrow{\text{reduction}} CO_2 + \underset{(\text{Aldehyde})}{R\cdot CH_2\cdot CHO} \rightarrow \underset{(\text{Alcohol})}{R\cdot CH_2\cdot CH_2OH}$$

The alcohols may be obtained from the α-amino acids *in vitro* by first oxidizing with hydrogen peroxide or some similar reagent and then reducing the aldehyde thus formed with sodium amalgam or zinc and acetic acid.

Ehrlich and Pistschimuka have shown that yeast and other fungi can utilise the nitrogen of amines with liberation of alcohols. Hydroxyphenylethylamine for example gave tyrosol, while isoamyl alcohol was obtained from amylamine. It would appear therefore that an alternative method for the production of alcohols from amino acids, not involving the formation of α-ketonic acids may be represented as follows:

$$R\cdot CH_2(NH_2)\cdot COOH \rightarrow R\cdot CH_2\cdot NH_2 \rightarrow R\cdot CH_2OH.$$

The formation of amines from amino acids (Reaction IV) may be readily imitated *in vitro* by simply heating the amino acid to a high temperature. Leucine, for example, gives isoamylamine:—

$$\begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!>\!CH\cdot CH_2\cdot CHNH_2\cdot COOH \rightarrow \begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!>\!CH\cdot CH_2\cdot CH_2NH_2$$

The decomposition of carboxylic acids (Reaction V) can usually only be effected *in vitro* by indirect methods. But aspartic and glutamic acids may be directly reduced to propionic and butyric acids respectively by heating to high temperatures with hydriodic acid (Kwisda). The same reaction is effected by bacteria.

O. Emmerling and Abderhalden and Y. Teruuchi showed that ammonium oxalate was formed by *Aspergillus niger* when grown in solutions of various α-amino acids, including glycine, alanine, aspartic acid, glutamic acid, proline and certain polypeptides. Leucine, phenylalanine, the diamino acids and the carbohydrates do not give oxalic acid under similar conditions. The mechanism of oxalate formation is unknown.

The principal decompositions of the common amino acids by micro-organisms are as follows:—

Glycine and Alanine are relatively resistant to bacterial decomposition (Nencki), but Brasch determined the formation of acetic and propionic acids respectively. Alanine may undergo fermentation with formation of ethyl alcohol (Ehrlich).

Serine is stated by Brasch to yield propionic and formic acids on anærobic decomposition.

Creatine is probably the mother-substance of methylguanidine but direct experiments with the pure amino acid are apparently lacking.

Creatinine was found by Ackermann to give N-methylhydantoin and sarcosine.

Cysteine is readily decomposed by micro-organisms yielding sulphuretted hydrogen and in some cases methylmercaptan, diethyl sulphide and thiosulphates. The reactions in the case of this amino acid are unusually complex (Wohlgemuth, Nencki).

Valine when acted upon by putrefactive organisms may yield isovaleric acid by reduction, and also an amine which is probably isobutylamine (Neuberg and Karczag). It may also undergo alcoholic fermentation with formation of isobutyl alcohol (Ehrlich).

Leucine was found by Nencki to give isovaleric acid. Barger found isoamylamine among the putrefactive products of meat, the base no doubt being derived from leucine. Leucine yields isoamyl alcohol on fermentation with yeast (Ehrlich).

Isoleucine probably yields *d*-methylethylpropionic acid by reduction since Neuberg and Rosenberg identified this acid together with *d*-valeric acid among the products of the putrefaction of caseinogen. On alcoholic fermentation it yields dextro-amyl alcohol (Ehrlich).

Aspartic Acid may yield acetic and formic acids by oxidation, propionic and succinic acids by reduction, and may part with carbon dioxide to form β-alanine (Ackermann, Borchardt, A. Harden, Neuberg and Cappezzuoli, E. & H. Salkowski).

Glutamic Acid undergoes bacterial decomposition in similar fashion to aspartic acid. The following products have been identified: Butyric, acetic, formic acids; succinic acid and γ-aminobutyric acid (Ackermann, Borchardt, Brasch, Neuberg). On alcoholic fermentation glutamic acid yields succinic acid (Ehrlich).

Proline has been shown by Ackermann to yield δ-aminovaleric acid through opening of the pyrrole ring by reduction. Neuberg has confirmed this observation and also observed the formation of normal valeric acid.

Ornithine may part with carbon dioxide yielding tetramethylenediamine (Ellinger) and may also give δ-aminovaleric acid by reduction and loss of ammonia (Ackermann).

Lysine similarly yields pentamethylenediamine (Ellinger).

Arginine on decomposition by bacteria may yield inactive ornithine and hence tetramethylenediamine and δ-aminovaleric acid (Ackermann).

Agmatine,

$$H_2N-\overset{\overset{\displaystyle NH}{\|}}{C}-NH\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH_2\cdot NH_2,$$

is also probably derived from arginine by bacterial action.

Histidine has been shown by Ackermann to yield β-iminazolepropionic acid by bacterial reduction and also the physiologically active base, β-iminazolethylamine. Organisms of the coli group have been shown by Raistrick to act on histidine with formation of urocanic acid. This production of an unsaturated acid is of considerable interest. Hirai has obtained iminazolyllactic acid from histidine by the action of B.proteus.

Phenylalanine yields a variety of products of bacterial decomposition. Phenylpropionic acid is obtained by reduction, phenylethylamine is obtained through loss of carbon dioxide, while phenylacetic acid and benzoic acid are products of oxidation (E. Baumann, Nencki, Salkowski, Barger and Walpole). Phenyllactic acid has also been identified by Amatsu and Tsudji. On alcoholic fermentation phenylalanine yields benzyl alcohol (Ehrlich).

Tyrosine. The following products have been identified: p-hydroxyphenylpropionic acid, p-hydroxyphenyllactic acid, tyrosol, p-hydroxyphenylacetic acid, p-hydroxyphenylethylamine, p-cresol and phenol (Weyl, Baumann, Nencki). E. and H. Salkowski consider that phenylpropionic acid may be formed from tyrosine by reduction both in the nucleus and the side-chain. This observation has not been confirmed, although Traetta-Mosca has described an organism which he states is capable of effecting the complete decomposition of tyrosine with intermediate formation of p-hydroxyphenylpropionic acid, benzoic acid and benzene. The identification of the benzene was inadequate, however. Tsudji has made the curious observation that B.proteus gives *d*-p-hydroxyphenyllactic acid when acting on either *d*- or *l*-tyrosine. B.subtilis on the other hand gives *l*-p-hydroxyphenyllactic acid from both active forms of tyrosine. It appears probable that the tyrosine is first converted into the optically inactive α-ketonic acid which is then reduced asymmetrically in opposite senses by the two microorganisms. On fermentation with yeast tyrosine yields p-hydroxybenzyl alcohol (tyrosol) (Ehrlich).

Tryptophan undergoes decomposition by bacteria in much the same way as tyrosine. The following products which had long been obtained from the products of the putrefaction of proteins were obtained from tryptophan by Hopkins and Cole: indolepropionic acid, indoleacetic acid, scatole and indole.

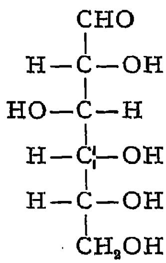
CHAPTER IV.

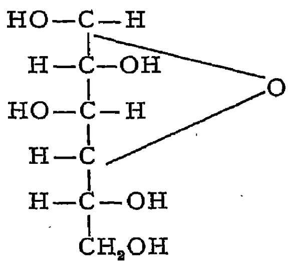
THE CARBOHYDRATES.

The sugars containing six or twelwe carbon atoms which commonly occur in animal or vegetable organisms furnish a very readily available source of energy for the animal body. It has been long known that tissues such as muscle in which active metabolism is in progress may obtain a large part of their energy by the combustion of sugars with liberation of carbon dioxide. Our knowledge of the mechanism of the chemical changes has been materially increased in recent years, but is still obscure as regards many of the fundamental details.

With the possible exception of the pentoses, the various carbohydrates contained in the foodstuffs are believed to yield chiefly glucose in the processes of digestion, absorption and assimilation. It follows therefore that the problems of carbohydrate metabolism chiefly centre around the chemical changes which glucose may undergo. The agents concerned with the oxidation of the sugars in the body are practically unknown. A great deal of work has been done on the subject of "glycolysis", the term implying the disintegration of the sugar molecule. Many dead tissues, especially certain combinations of tissue plasma and extracts, such as muscle and pancreas have been believed capable of effecting the decomposition of glucose. But Levene and Meyer have shown that the apparent decomposition of glucose in such experiments is in reality due to the formation of polysaccharides, and it is probable that a similar synthesis is concerned in many other experiments in which a disappearance of glucose has been inferred simply from a change in reducing power and optical rotation. It is of interest to recall that a similar synthesis of polysaccharides is constantly observable in the alcoholic fermentation of glucose by yeast. The physiological aspects of animal glycolysis are outside the scope of this chapter in which the structural relations of the sugars and their decomposition products are alone considered, but it may be noted that active glucose metabolism only seems to occur in the presence of living or surviving cells.

Structure of glucose. The newer investigations into the detailed structure of the sugars have shown that 'glucose' instead of being a single chemical individual exhibiting mutarotation in solution, exists in a variety of interconvertible isomeric forms possessing marked differences in chemical as well as physical properties. These discoveries have introduced important complications into the study of glucose metabolism, the existence of which are recognised but not yet understood. The old formula for glucose (I) representing it as a hydroxyaldehyde is still used a good deal by physiological chemists, although it is almost certain that not more than minute amounts of a substance with this structure, exist in any glucose solution. It is now believed that ordinary glucose consists mainly of a balanced mixture of two isomeric forms, α and β, which are interconvertible, and which both contain a butylene oxide ring, but no aldehyde group (II. III). These two forms correspond to the α- and β-series of alkyl-glucosides obtained from "glucose" when treated with an alcohol and hydrochloric acid. More recently Fischer obtained a third methyl-glucoside which Irvine, Fyffe and Hogg showed to be a mixture of two isomeric forms corresponding to two new forms of glucose (IV. V). These new forms of glucose and their derivatives are known as γ-compounds and contain the ethylene oxide ring. The space formulae for the aldehyde, two butylene oxide, and two ethylene oxide forms of glucose are given below:


I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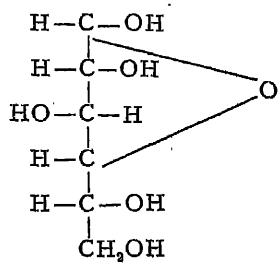
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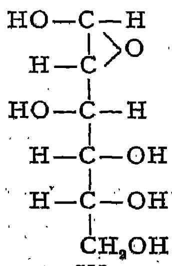
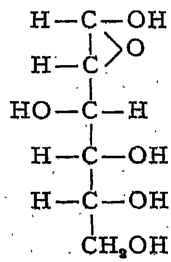

IV


V

The γ- or ethylene oxide forms of glucose and their derivatives are characterised by extraordinary instability towards oxidising agents, such as permanganates, and undergo various condensations with much greater speed than the α- or β-compounds. Armstrong and Hilditch have brought forward evidence suggesting that when ordinary glucose solutions are oxidised in acid solution with potassium permanganate, it is the small amount of γ-glucose present in the solution which reduces the permanganate instantaneously, and that further small amounts of the γ-isomeride are then produced to take the place of that destroyed. Whatever the first interpretation of these results may be, it is almost certain that small amounts of γ-glucose may co-exist with the α- and β-forms in glucose solutions and the high reactivity of the γ-forms would certainly suggest that they may well be concerned in various biochemical transformations. Furthermore, evidence is now available demonstrating an ethylene oxide structure for both the glucose and fructose nuclei in sucrose. A most suggestive study of the stereochemical changes undergone by equilibrated solutions of glucose and other sugars, in the alimentary canal has been published by Hewitt and Pryde. They find that the glucose solution, which was previously in stable equilibrium as regards the various forms of glucose present (chiefly α and β) on introduction into the intestine undergoes a rapid downward mutarotation. After withdrawal from the intestine the reverse change takes place but more slowly. After proving that the change observed is not due to preferential absorption of α-glucose or production of β-glucose, the conclusion is reached that contact with the intestinal mucosa probably effects the production of the γ- or ethylene oxide form of glucose in excess of any amount normally present in the original glucose solutions. That the highly reactive γ-glucose may be the preferred form for metabolic change must be regarded as not unlikely, although a definite decision as to this will probably not be very easy to secure.

Further evidence of the extraordinary capacity of the sugars to undergo intramolecular and other rearrangements is furnished by the experiments of Nef and Glattfield. It is found that each hexose dissolved in alkaline solution gives a mixture of six isomeric hexoses in addition to a mixture of sugars containing 2, 3, 4 and 5 carbon atoms. The evidence for this statement is largely based on an extensive study of the products of oxidation of glucose and other sugars in alkaline solution using either air or hydrogen peroxide as the oxidizing agent. It is interesting to note that Löb and Beysel have

shown that the oxidation of glucose by hydrogen peroxide is catalytically accelerated by phosphates. The effect seems to be relatively specific and is not simply a function of the hydrogen ion concentration. What rôle, if any, the phosphates play in the degradation of glucose in the animal body is not known, though their function in alcoholic fermentation is now well established.

Glucose-Lactic acid Relationships. It has long been recognised that the hexoses containing six carbon atoms could be converted into substances containing three carbon atoms by a variety of processes, while conversely the synthesis of certain hexoses could be effected by condensation of two molecules of certain substances containing three carbon atoms. The formation of lactic acid by the action of alkali upon glucose and the synthesis of fructose and sorbose by the action of dilute alkali upon glyceric aldehyde (Schmitz) may be cited as examples of these changes. It appears that reactions of this type are of great importance in carbohydrate metabolism.

Thus, glucose, mannose and fructose solutions all give significant amounts of d-lactic acid when incubated with sterile kidney tissue or with leucocytes (Levene and Meyer). Similarly whole blood of certain species is able to convert a small amount of glucose into lactic acid (Kondo; Kraske), a reaction which van Noorden jun. has shown to be brought about solely by the corpuscles and not by plasma or serum. A relatively larger yield of lactic acid has been observed by Embden and his co-workers, when a surviving liver rich in glycogen, and hence capable of yielding glucose, is submitted to perfusion. Similarly, addition of glucose or fructose and probably d-sorbose to the perfusing fluid results in d-lactic acid formation. It should be noted that fructose and d-sorbose are both apparently converted into glucose on perfusion through the liver under appropriate conditions, hence it may be inferred with considerable probability that in each case the d-lactic acid originates from glucose. Conversely it is known that lactic acid may lead to glycogen formation in the normal animal body and when given to a diabetic animal it is practically quantitatively converted into glucose (Mandel and Lusk and others). It is worth noting that this conversion of lactic acid into glucose takes place equally completely whether the acid is the d-, l-, or inactive variety. It is thus clear that lactic acid must be regarded as one of the most important substances concerned in the intermediary metabolism of the carbohydrates. Quantitatively its significance far outweighs that of any of the other acids derivable from sugars which will be referred to later.

$$\begin{array}{ccccc} & & \text{sorbose} & & \\ & & \downarrow & & \\ \text{Glycogen} & \rightleftarrows & \text{glucose} & \rightleftarrows & \text{lactic acid} \\ & & \uparrow & & \\ & & \text{fructose} & & \end{array}$$

The mechanism of the formation of lactic acid from glucose and *vice versa* and the subsequent fate of lactic acid in the body are obviously matters of great importance and in recent years noteworthy advances have been made towards the solution of the problem although many important details await solution. It is fairly certain that the interconversion of glucose and lactic acid is quantitative, that is to say that each molecule of glucose ($C_6H_{12}O_6$) yields two molecules of lactic acid ($C_3H_6O_3$). This is inferred not only from the simple relation existing between the empirical formulae of lactic acid and glucose but also from the fact that in well controlled biochemical experiments involving both degradation and synthesis of glucose, the amount of lactic acid produced or disappearing has been found to correspond closely with the above ratio. An inspection of the structural formulae for glucose and lactic acid show that complex rearrangements are necessary for the production of the latter substance. Light on the possible mechanisms involved in the reactions has been obtained by the study of the action of alkali upon glucose under various conditions. As already stated lactic acid is formed by the action of alkali upon glucose but by varying the conditions other products may be obtained leading to the inference that the following substances are to be considered potential precursors of lactic acid, namely glyceric aldehyde (I), dihydroxy acetone (II) and pyruvic aldehyde (methyl glyoxal) (III):

$$\begin{array}{lll} CH_2OH & CH_2OH & CH_3 \\ | & | & | \\ CHOH & CO & CO \\ | & | & | \\ CHO & CH_2OH & CHO \\ \text{I} & \text{II} & \text{III} \end{array}$$

The work of Pinkus, Nef, Wohl and others has led to the belief that in the production of lactic acid from glucose *in vitro*, glyceric aldehyde is first formed (1); possibly but much less probably dihydroxyacetone may also be produced. These latter substances may then furnish pyruvic aldehyde through less of water (2). The pyruvic aldehyde then takes up a molecule of water with formation of lactic acid: —

$$\begin{array}{lll} (1) & CH_2OH\cdot(CHOH)_4\cdot CHO & \rightarrow 2\,CH_2OH\cdot CHOH\cdot CHO \\ (2) & CH_2OH\cdot CHOH\cdot CHO & \rightarrow CH_3\cdot CO\cdot CHO \\ (3) & CH_3\cdot CO\cdot CHO & \rightarrow CH_3\cdot CHOH\cdot COOH \end{array}$$

The main evidence for this theory is as follows:

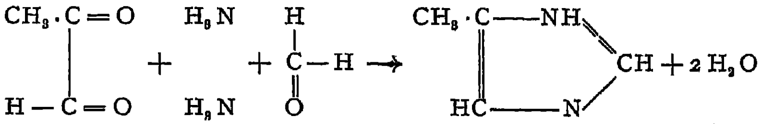
(a) Glucose when acted upon by alkali in the presence of phenylhydrazine yields the osazone of pyruvic aldehyde (Pinkus). Pyruvic aldehyde may be detected in distillates obtained from glucose and disodium hydrogen phosphate solutions (Dakin and Dudley). Fernbach and Schoen state that pyruvic aldehyde is produced by the action of dilute sodium carbonate on glucose.

(b) Pyruvic aldehyde on treatment with alkali readily yields lactic acid in practically quantitative amount. The same changes occur but less completely on long heating with water (Denis).

(c) Dihydroxyacetone on distillation with dilute sulphuric acid yields pyruvic aldehyde (Pinkus); while on treatment with ammonia and phenylhydrazine it yields the osazone of pyruvic aldehyde (Neuberg).

(d) Glyceric aldehyde, like glucose gives lactic acid when treated with alkali though less smoothly than does pyruvic aldehyde (Nef).

(e) The synthesis of methyliminazole from glucose and ammonia in the presence of zinc oxide is readily explained on the assumption that pyruvic aldehyde and formaldehyde are formed from glucose (Knoop and Windaus): —



(f) The formation of saccharines from glucose is probably due to synthesis from glyceric aldehyde and lactic acid as intermediate products.

The results collected above as to the mechanism of lactic acid formation from glucose *in vitro* clearly invited consideration of the possible occurence of similar reactions *in vivo*. Accordingly, each of the three aforementioned intermediary bodies namely glyceric aldehyde, dihydroxyacetone and pyruvic aldehyde has been carefully examined as to its biochemical behaviour. The experiments with the first two substances have been mainly due to Embden and his co-workers, while Dakin and Dudley and also Neuberg have been concerned with the latter. Before considering some of the experimental results thus obtained it may be said that Embden definitely rejects the idea that pyruvic aldehyde may be a product of the intermediary metabolism of glucose and related sugars but strongly adheres to the view that glyceric aldehyde is the main intermediate product between glucose and lactic acid, while dihydroxyacetone was at first assigned a sub-

ordinate rôle but now appears to be regarded more favourably. Dakin and Dudley on the other hand have produced a good deal of evidence which suggests to them that pyruvic aldehyde may be seriously considered as an intermediate product in the conversion of glucose into lactic acid possibly by way of glyceric aldehyde. Recently Embden and his co-workers have produced evidence indicating that the formation of lactic acid from carbohydrates in preceded by a union of the latter substances with phosphoric acid. The substance so formed has been named 'lactacidogen' and it appears to be similar but not identical with the known hexosephosphoric acids. It is likely that phosphates may be of importance in animal carbohydrate metabolism as is the case with ordinary alcoholic fermentation. The investigations have not yet proceeded sufficiently far to warrant very definite conclusions.

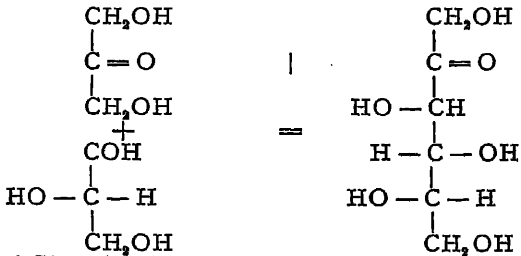
The main evidence leading to the belief that glyceric aldehyde is concerned in glucose catabolism may be summarised as follows:

Miss Smedley found that when glyceric aldehyde is incubated with liver tissue it completely disappears in the course of a few hours, but the products of the reaction were not determined. Under similar conditions dihydroxyacetone and glucose were virtually unaffected. The reaction was more completely investigated by Embden and his colleagues. First of all it was shown by Embden, Baldes and Schmitz that inactive glyceric aldehyde is rapidly converted into lactic acid by fresh blood corpuscles or by perfusion through the liver. But the acid isolated proved to be a mixture of inactive lactic acid with excess of *l*-lactic acid. This result is somewhat surprising since the lactic acid naturally occuring in the animal body appears to be exclusively the dextro variety.[1] On the other hand dihydroxyacetone was much less readily changed by blood corpuscles and furnished d-lactic acid. It was inferred that the laevo-rotation of the lactic acid obtained from glyceric aldehyde was due to the fact that synthetic inactive glyceric aldehyde had been used in the experiments. The conclusion drawn at first was that in the natural formation of d-lactic acid from glucose two molecules of optically active glyceric aldehyde of like configuration are produced and the latter are directly converted into d-lactic acid. Actual experiments with optically active glyceric aldehyde appear to be still lacking. An important side reaction was observed in the formation, during liver perfusion, of small amounts of glycerol from both glyceric aldehyde and dihydroxyacetone, thus

[1] Under special circumstances rabbits have been observed to exrete inactive lactic acid in the urine but this hardly affects the above statements.

making probable the origin of the former substance from glucose in the living animal.

Having demonstrated the formation of l-lactic acid from inactive glyceric aldehyde and d-lactic acid from dihydroxyacetone in the surviving liver, attention was next directed to the possibility of detecting hexose synthesis from the two trioses just mentioned. A curious result was obtained namely that while dihydroxyacetone which is necessarily optically inactive proved to be a vigorous producer of d-glucose in the glycogen-poor liver, inactive glyceric aldehyde gave chiefly d-sorbose, a ketose isomeric with fructose. Later experiments by Embden and Griesbach in which the behavior in the liver of d-sorbose was investigated led to a doubtful production of d-lactic acid in one out of three experiments, but a definite partial conversion into d-glucose. The formation of d-sorbose from inactive glyceric aldehyde is pictured by Embden, Schmitz and Wittenberg as the result of the condensation of one active from of glyceric aldehyde, namely the l-form with dihydroxyacetone:



l-Glyceric Aldehyde d-Sorbose

If this interpretation be correct the possibility of the conversion *in vitro* of glyceric aldehyde into dihydroxyacetone has to be admitted and from Embden's later papers it would appear that he is inclined to ascribe a much more important rôle to dihydroxyacetone as a precursor of d-lactic acid, glucose and glycerol than was previously considered probable. In harmony with the view that glyceric aldehyde and dihydroxyacetone may participate in glucose metabolism it has been found that under suitable experimental conditions a fairly complete conversion of both of these substances into glucose takes place in the phlorhizinised diabetic dog (Sansum and Woodyatt; Ringer and Frankel). Further, Parnas has observed the formation of glycogen from glyceric aldehyde in the tortoise liver.

The facts, other than those already cited, which entitle pyruvic aldehyde to consideration as a possible metabolite of glucose, may be summarised as follows: In the first place it is possible actually to isolate small amounts of pyruvic aldehyde from glucose when the latter

is treated with feebly alkaline salts. In this respect it differs from both glyceric aldehyde and dihydroxyacetone, though the difference is not actually as significant as might appear. Secondly, dilute solutions of lactic acid on digestion with p-nitrophenylhydrazine give small amounts of the very sparingly soluble p-nitrophenylosazone of pyruvic aldehyde. It would thus appear as if slight dissociation of lactic acid into pyruvic aldehyde had occurred:

$$CH_3 \cdot CHOH \cdot COOH \rightarrow CH_3 \cdot CO \cdot CHO + H_2O$$

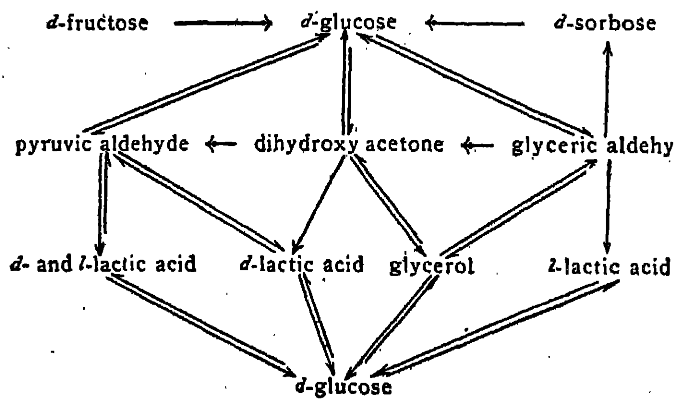
The reaction is a complex one and several other products are formed (Dakin and Dudley).

Next it has been shown that enzymes which have been named "glyoxalases" exist in most tissues and that these convert pyruvic aldehyde (methyl glyoxal) into lactic acid with extraordinary speed, provided the acid is neutralised as it is formed. An idea of the activity of the enzyme may be judged from the fact that a crude aqueous extract of liver containing less than a gram of organic matter can form several grams of lactic acid in the course of a few minutes. Curiously enough the lactic acid formed usually but not always contains an excess of the laevo compound, though the proportion of *l* to *d* varies considerably. It will be noted that a similar difficulty arises from the formation of the l-acid as was encountered with glyceric aldehyde. The difficulty is however not an insuperable one for, when working with the analogous constituted phenyl glyoxal, it was found that from the same enzyme solution mandelic acid containing an excess of the *l* compound could be obtained at first, followed by the production of an acid containing an excess of the dextro variety. Whether this is due to the action of more than one enzyme or to the slow conversion of the glyoxal into a hydrate or some other isomeric form which then gives the *d*-acid is not known. The glyoxalases are found in most animal tissues except the pancreas which curiously enough contains a thermolabile substance, presumably of enzyme character which promptly inhibits the action of glyoxalase. The known relation of the pancreas to disturbances of glucose metabolism makes this observation of interest.

Finally, it has been found that not only pyruvic aldehyde but also l-lactic acid, as well as the d-lactic acid normally present in the animal body are all equally and practically quantitatively convertible into d-glucose in the phlorhizinised diabetic animal. Since it is impossible to picture the conversion of l-lactic acid into d-glucose without the intervention of reactions involving the loss of asymmetry and hence optical activity of the lactic acid, the conclusion appears

inevitable either that a necessarily optically inactive symmetric intermediate product, such as either pyruvic aldehyde or dihydroxyacetone, is an intermediate stage in the interconversion of glucose and lactic acid, or less probably, that some active form of an intermediary product is equally derivable from d or l-lactic acid, a reaction which would involve some form of optical inversion of a type not yet encountered in animal metabolism.

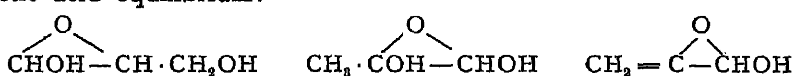
By combining the results obtained by experiments *in vitro* and *in vivo*, especially those occurring in the perfused liver or the diabetic organism it appears that the equilibria or reactions shown in the following diagram have been fairly satisfactorily demonstrated. The actual isolation of glyceric aldehyde and dihydroxyacetone from glucose has not been accomplished but their formation, transitorily at any rate, can hardly be doubted from the results of the experiments on the action of alkali on glucose already referred to.



The above representation is non-committal as regards the actual course of glucose metabolism. For this purpose the reader will be able to construct a diagram according to his own predilection showing any or all of the possible metabolites or may reject any such attempts at rigid representation.

It appears to the writer that at the present time it is impossible to reach a definite decision as to the rôle of glyceric aldehyde, dihydroxyacetone and pyruvic aldehyde in glucose metabolism. Most of the evidence is indirect and complete proof, isolation of the intermediate products, does not appear probable of accomplishment. As will have been noted there are certain difficulties in the acceptance of any of the proposed hypotheses. It would not be surprising in

the writer's estimation if eventually some highly reactive substance or substances of the type of the recently described γ-glucose may prove to be concerned in the glucose-lactic acid interconversion. Thus for example glyceric aldehyde which is only known in one structural form might exist in a more labile ethylene oxide modification (I); while pyruvic aldehyde, known only in one simple form and a polymeric modification, may exist theoretically in no less then eight different optically inactive forms including various hydrates (Neuberg). It is noteworthy that two of these forms contain optically active carbon atoms and also the ethylene oxide ring (II. III). If the existence of some such active modification of pyruvic aldehyde should be determined, it might clear up most of the remaining difficulties in connection with the understanding of the glucose-lactic acid equilibrium:



I II III

Reference should be made to experiments by Sansum and Woodyatt, in which they determinad the tolerance of dogs for *d-l*-glyceric aldehyde when given intravenously. They find that the triose begins to appear in the urine when the dose exceeds 0.15 gram per hour. This tolerance is only about one sixth of that found for glucose under similar conditions. From this and other reasons these authors state that they "believe that this particular change of glucose into glyceric aldehyde does not constitute an important step in its disintegration in the living body and that conceptions of the break down of glucose in which the process is depicted as though it preceeded from one fixed molecule to another are fundamentally inadequate". The writer, on the whole, is inclined to doubt the validity of the inference drawn from the lower tolerance showed by dogs for glyceric aldehyde as compared with glucose, though recognising that their views are entitled to great consideration. It would appear that the experiment would be more convincing if we knew that the blood represented the controlling seat of the glucose-lactic acid equilibrium.

Objection to current views as to the glucose-lactic acid equilibrium have also been brought forward by Parnas and Baer. They object to the view that lactic acid can be directly transformed into glucose, since the reaction is endothermic to the small extent of 25 calories. They have therefore constructed a scheme based on experiments with diabetic animals in which 3 mols. lactic acid furnish

1 mol. glucose and the synthesis of the latter is pictured as taking place via glyceric acid and β-hydroxypyruvic acid and glycollic aldehyde, all the reactions being exothermic. But the capacity of the body to effect endothermic reactions is well established and as Embden has pointed out the energy relations between glucose and lactic acid are in fact in harmony with the idea of their convertibility. It appears to the writer that the scheme put forward by Parnas and Baer requires much greater experimental evidence before it can compete with more generally accepted views.

The subsequent fate of lactic acid derived from glucose is discussed in the section on hydroxy acids. The oxidation of lactic acid to pyruvic acid, brings the former substance into biohemical relationship with alanine, acetaldehyde, alcohol, acetoacetic acid and probably acetic acid (Cp. Chapter III).

Glucuronic Acid. Certain other types of carbohydrate decomposition not involving the production of lactic acid demand consideration. More than forty years ago Wiedemann and Schmiedeberg and Meyer found that when camphor was given to animals it was excreted in combination with a substance related to the carbohydrates. The same substance, glucuronic acid, has since then been found to be present in small amounts in the blood, in the liver, and in normal urine. It is excreted in large amounts in combination with various aromatic alcohols, phenols, ketones, and acids when these substances are given to animals. Apparently the acid is never excreted in the free state, but always in combination. The close structural relationship between glucuronic acid (II) and glucose (I) made it appear probable that glucuronic acid was derived from the oxidation of the sugar:—

CHO	CHO	COOH	COOH
HCOH	HCOH	HCOH	HCOH
HOCH	HOCH	HOCH	HOCH
HCOH	HCOH	HCOH	HCOH
HCOH	HCOH	HCOH	HCOH
CH_2OH	COOH	COOH	CH_2OH
d-Glucose	Glucuronic acid	d-Saccharic acid	Gluconic acid
I	II	III	IV

Evidence of the formation of glucuronic acid from sugar was furnished by the following experiments of Paul Mayer, who found that rabbits after prolonged starvation, and hence containing extremely little stored glycogen, furnished very little glucuronic acid

on administration of camphor, but on subcutaneous administration of camphor and glucose the normal excretion of the glucuronic acid derivative of camphor was at once obtained.

The observation of Hildebrandt that the toxicity of thymotin-piperidide was diminished by simultaneous administration of large quantities of glucose, presumably because the sugar furnished an additional supply of glucuronic acid which on combination with the foreign substance diminished its toxicity, may be regarded as additional evidence for the occurence of this change. Moreover, in certain cases of artifically induced dyspnœa in animals an excretion of glucose and glucuronic acid was observed by Mayer. The presence of the latter substance was ascribed to impaired power of oxidation on the part of the animal. The results of these latter experiments have not been adequately confirmed.

Assuming that glucuronic acid represents the first step in the oxidation of glucose, further oxidation might be expected to give saccharic acid (III). Now Mayer stated that when large amounts of glucose, glucuronic acid, or saccharic acid, were given to rabbits a small but varying amount of oxalic acid was excreted in the urine. The oxidation of glucuronic acid to oxalic acid was also observed to take place in an excised liver. It appeared conceivable that sugar, at least in part, might undergo oxidation in the animal body so as to yield successively glucuronic acid and oxalic acid, with possible intermediate formation of saccharic acid.

The results of Mayer have not been confirmed and are apparently mostly erroneous. Biberfeld has shown that glucuronic acid whether given to rabbits and dogs intravenously or subcutaneously is excreted unchanged practically quantitatively. Gluconic acid (IV) and saccharic acid are also excreted unchanged after the administration of small doses. Mayer's statements as to the formation of saccharic acid from gluconic acid when fed to rabbits could not be confirmed by E. Schott and apparently are incorrect according to the later investigations of Paderi. It would appear therefore that glucuronic acid does not represent a main product of carbohydrate metabolism and its formation in more than traces is probably dependent on the presence of other compounds, such as phenols, aromatic alcohols, ketones and aldehydes, with which it combines. This change represents a defensive mechanism rather than normal metabolism and serves to reduce the toxicity of the aromatic compounds. Furthermore when once formed, glucuronic acid appears to be resistant to further oxidation.

Until recently the direct oxidation *in vitro* of glucose to glucuronic acid had not been accomplished, as under most conditions the primary alcohol group is not attacked, but rather the other terminal group so that gluconic acid is usually formed. But by gradual oxidation of glucose in neutral solution with dilute hydrogen peroxide, Jolles has been able to determine the production of glucuronic acid in small amounts. Glucuronic acid in turn is readily oxidised to saccharic acid by bromine water (Thierfelder), while *d*-saccharic acid on treatment with nitric acid gives tartaric, racemic and oxalic acids (Hornemann). But as stated before these latter reactions do not appear to have biochemical counterparts.

Brief reference may be made to the experiments of Wirth showing that saccharic, gluconic and mucic acids when added in sufficient quantity to blood supplying perfused dog's livers may lead to a marked formation of acetoacetic acid. The reaction is probably of little biochemical significance but since the action of glucose under similar conditions is to reduce acetoacetic acid formation, it may be taken as confirmatory evidence of the non-production of these acids in normal glucose metabolism.

Ethyl Alcohol. The fact that it is possible to isolate small quantities of ethyl alcohol by the distillation of aqueous extracts of animal tissues has led at times to the belief that sugars might undergo alcoholic fermentation in the animal body. Harden and McClean examined the question carefully and concluded that under aseptic conditions no alcohol is produced. It appeared that the minute amount of alcohol found in fresh animal tissues originated from intestinal fermentation. A later experiment by A. E. Taylor does not support this view. A dog which had starved for a day prior to operation had the whole of its alimentary tract removed and eighteen hours after operation the animal was killed and the muscles at once examined for alcohol. A small but definite amount was satisfactorily identified. It appears therefore that small amounts of alcohol may be formed in the tissues, presumably from glucose, and independently of bacterial action. Ethyl alcohol cannot however be regarded as one of the chief metabolic products of glucose in the animal body.

During the last ten years great progress has been made in unravelling the course of events leading up to the alcoholic fermentation of glucose by yeast. It seems well established now that pyruvic acid is undoubtedly an intermediate product and that it is converted into carbon dioxide and acetaldehyde and that the latter is then reduced

to ethyl alcohol. The production of pyruvic acid — a substance which may be regarded as an oxidation product of glucose — only appears possible when an acceptor for hydrogen is present and normally acetaldehyde subserves this function.

$$CH_3 \cdot CO \cdot COOH \rightarrow CH_3 \cdot CHO + CO_2 \xrightarrow[+2H]{} CH_3 \cdot CH_2OH + CO_2$$

Whether similar reactions to these are responsible for the production of alcohol in the animal body is not definitely known, but at least it is known that pyruvic acid may yield acetaldehyde in the body and that the latter may be converted into ethyl alcohol and acetic acid, *in vivo*, through the Cannizzaro reaction.

Pentoses.—Reference must be made to the possibility of a fourth type of carbohydrate decomposition which may possibly be of importance in connexion with sugar catabolism. Walther Löb has shown in a long series of papers that the depolymerization of glucose does not necessarily result in the formation of two molecules of substances containing three carbon atoms, but that under certain circumstances, e.g. by very dilute alkali, or by electrolysis, or certain other methods of oxidation, a more gradual decomposition may occur leading first of all to the formation of a pentose and formaldehyde. By successive depolymerization the sugar molecule may be further resolved with formation of additional molecules of formaldehyde and Löb refers the production of many substances, derived from glucose by biochemical reactions, to their synthesis from formaldehyde and other products of partial depolymerization. It is not clear that reactions such as these play any part in oxidations in the animal body although they do present striking analogies with reactions taking place in the vegetable organism. The possible formation from hexoses of the pentoses which are found combined in the nucleic acids of animal cells may be correlated with reactions of this type. The derivation from any of the common hexoses of d-ribose (V), the pentose which Levene and Jacobs have obtained from a number of nucleic acids, would necessitate stereochemical rearrangements, but on the other hand it may possibly be formed synthetically from formaldehyde, or other simple products of the depolymerization of the hexoses. Neuberg has suggested that the inactive arabinose (II) which he states is excreted in the peculiar metabolic abnormality known as pentosuria, is derived from galactose (I).[1] As an actual example of the

[1] Recent experiments by Raper throw doubt on the excretion of arabinose in pentosuria. The sugar in pentosuric urine appears to resemble ribose more closely than any other of the known pentoses.

biochemical conversion of hexose to pentose it is of interest to note that glucuronic acid (III) derived from d-glucose may be converted into l-xylose (IV) by means of putrefactive micro-organisms. The transformation of hexoses to pentoses in the animal body must be regarded as probable, although unproven.

CHO		CHO			CHO
HCOH	CHO	HCOH	CHO	CHO	$CHNH_2$
HOCH	HCOH	HOCH	HCOH	HCOH	HCOH
HOCH	HOCH	HCOH	HOCH	HCOH	HCOH
HCOH	HOCH	HCOH	HCOH	HCOH	HCOH
CH_2OH	CH_2OH	COOH	CH_2OH	CH_2OH	CH_2OH
d-Galactose	l-Arabinose	Glucuronic acid	l-Xylose	d-Ribose	Glucos-amine
I	II	III	IV	V	VI

At one time it was thought that glucosamine (VI) was an important link connecting the proteins on the one hand and carbohydrates on ther other, but E. Fabian's experiments showed that glucosamine is not very readily oxidized in the body; when given subcutaneously much was excreted unchanged in the urine. The manner of its oxidation is unknown.

Brief mention must be made of the fate of the pentoses in the animal body. The pentosans, i.e. the complex polysaccharides which on hydrolysis yield the pentoses, are most important constituents of the food of herbivorous animals. The questions concerning the mechanism of the hydrolysis of these substances are complex, but it is certain that in one way, or another, large quantities of pentoses undergo absorption. In general the pentoses are not so readily utilized in the body as the hexoses (cf. V. Jaksch and Salkowski), and when moderate amounts are administered to animals a considerable proportion may be excreted in the urine. Thus, on feeding 10-15 grms. of l-arabinose to starving rabbits about 18 per cent. was excreted unchanged in the urine. Cremer, by respiration experiments, showed that rhamnose, a methyl pentose, underwent oxidation in the body with formation of carbon dioxide, but the mechanism of the oxidation is entirely obscure. It appears, however, that under certain circumstances the pentoses may yield hexoses, i.e. the reverse process to that referred to above. Thus, it has been found that l-arabinose, l-xylose and rhamnose, when fed to starving rabbits, apparently may lead to the formation of ordinary glycogen, which on utilization would yield glucose (E. Salkowski, Cremer, and others).

Moreover, several cases have been observed in which the administration of pentoses to diabetics has led to the excretion of glucose, although at the time immediately preceding the experiment the excretion of glucose had been abolished (R. v. Jaksch, Lindemann and May, P. Bergell). It must not be forgotten, however, that according to von Jaksch, administration of large quantities of pentoses to diabetics increases the protein catabolism, and in any case it is quite possible that the excretion of glucose was caused indirectly.

The fact that it is apparently possible for pentoses and hexoses to undergo mutual interconversion, whatever the mechanism of the process may be, is of interest in connexion with Löb's theory of sugar synthesis and depolymerization.

Information concerning the biochemistry of diabetes in which the organism loses more or less completely its customary capacity to effect the oxidation of glucose must be sought in the special works on chemical pathology (e.g. Van Noorden's "Metabolism and Practical Medicine"). It is of importance to note that this failure to effect the oxidation of glucose does not extend to other derivatives of glucose, except in so far as some of them, such as lactic acid, may be converted into glucose.

Polyhydric Alcohols.—The polyhydric alcohols are so closely related to the carbohydrates that they may be conveniently considered in connexion with the sugars. Glycerol is the only one of these substances of much importance in animal metabolism. The simplest polyhydric alcohol, glycol,

$$CH_2 \cdot OH \cdot CH_2 \cdot OH,$$

so far as is known, is not formed in the animal body. When given to dogs, or rabbits, it is partly oxidized to oxalic acid (Pohl). Mayer found that when large doses were given to rabbits it was possible to isolate glycollic acid from the urine in the form of its phenylhydrazide. It is probable that glycollic and glyoxylic acids are intermediate products of the oxidation of glycol (Dakin). It is doubtful if more than a part of any glyoxylic acid formed is oxidized to oxalic acid.

$$\begin{array}{ccccccc} CH_2OH & & COOH & & COOH & & COOH \\ | & & | & & | & & | \\ CH_2OH & \rightarrow & CH_2OH & \rightarrow & CHO & \rightarrow & COOH \\ \text{(Glycol)} & & \text{(Glycollic acid)} & & \text{(Glyoxylic acid)} & & \text{(Oxalic acid)} \end{array}$$

Complete oxidation with intermediate production of formic acid is probably an alternative path for the oxidation of glyoxylic acid.

Glycerol is readily utilized in the animal body. When given to animals in large amounts, a part may be excreted unchanged, but

under most circumstances it is completely oxidized (Luchsinger, Nicloux, and others). Ordinarily the concentration of free glycerol in the body must be very small, but it is constantly being formed through the hydrolysis of fats.

Blood contains from 0·0017 to 0·0048 per cent. depending upon the species (Schmitz) but while these amounts are small it is constantly being produced by the hydrolysis of fats and in addition when necessary can be synthesised from the sugars.

The close relationship of glycerol to the sugars and to lactic acid is clear from the following observations:

On perfusion of dogs livers containing but little glycogen with blood containing glycerol it was found by Oppenheimer that limited formation of *d*-lactic acid occurred. It is natural to assume that either glyceric aldehyde or dihydroxyacetone is first produced by the oxidation of the glycerol. The reverse change was then demonstrated by Embden, Schmitz and Baldes who found that liver and kidney tissue could reduce glyceric aldehyde into glycerol, although lactic acid was the chief product. Similar experiments with dihydroxyacetone appear not to have been reported though Oppenheimer showed it was a source of glycerol for yeast. The relationship between glyceric aldehyde, dihydroxyacetone and glycerol is shown by the following formulæ

$$\begin{array}{lclcl} CHO & & CH_2OH & & CH_2OH \\ | & & | & & | \\ CHOH & \longrightarrow & CHOH & \longleftarrow & CO \\ | & & | & & | \\ CH_2OH & & CH_2OH & & CH_2OH \end{array}$$

Pyruvic aldehyde must also be regarded as a possible source of glycerol, although experiments in this direction are at present lacking. The tautomeric form, $CH_2 = COH \cdot CHO$, can readily be pictured as capable of reduction to glycerol.

The formation of *d*-glucose from glycerol was believed to take place to a small extent in the perfused liver by Emden, Schmitz and Wittenberg. But a much more complete conversion was observed by both Cremer and Luthje who found a large increase in glucose excretion to follow the administration of glycerol to diabetic animals.

The mutual interconversion of glucose and glycerol seems easily effected in the animal organism within the limits of metabolic requirements. It is probable that the main path of the normal catabolism of glycerol is by way of *d*-lactic acid.

The higher polyhydric alcohols of vegetable origin, erythritol $C_4H_6(OH)_4$, quercitol $C_6H_7(OH)_5$ and mannitol $C_6H_8(OH)_6$ when given

to animals in large amounts are in part excreted unchanged in the urine (Von Mering, Pohl, Luchsinger). Embden and Griesbach found that *d*-sorbitol gives *d*-lactic acid when perfused through the liver of a starving animal, while if the animal had been previously treated with phlorhizin, reducing sugars are formed, apparently *d*-fructose and *d*-glucose. Mannitol, dulcitol and inositol gave negative results. Careful metabolic experiments by Anderson have shown that inositol when given to dogs in doses of 2 grams per kilogram is slowly absorbed from the bowel and as much as 77 per cent. may be recovered unchanged from the excreta. When given to man, 0·5 gram per kilo, about nine per cent is excreted unchanged. The fate of the remainder is unknown. In general the higher polyhydric alcohols appear to be much less readily utilised than are the true carbohydrates.

In conclusion reference may be made to the fate in the body of two other substances closely related to glucose. The lactone of glucoheptonic acid, a substance readily synthesised from glucose by means of the cyanhydrin reaction, has been used as a substitute for glucose by diabetics and is known under the trade name of Hediosit. Experiments by Lenel have shown that when given by mouth either to normal or diabetic individuals 25-50 per cent. is excreted unchanged while when given intravenously it is quantitatively excreted. The substance is therefore of little or no value for the purpose it was intended for. The behaviour of glucal, an unsaturated glucose derivative recently obtained by Fischer, having the formula:

$$\overbrace{CH = CH \cdot CHOH \cdot CH}^{O} \cdot CHOH \cdot CH_2OH$$

has been examined by Balcar. The substance is not toxic and non-fermentable but is not completely catabolised when injected into dogs at the rate of 0·9 gram per kilo per hour. Its biochemical significance, if any, is obscure.

CHAPTER V.

THE PURINE DERIVATIVES.

The purine bases occur in two forms in the animal body—free and combined. The free purine bases, especially hypoxanthine, occur to a limited extent in certain tissues, especially muscle. The combined purine bases have a wider distribution. They are found in combination with phosphoric acid, a carbohydrate, and in some cases with bases of the pyrimidine group in the form of complex nucleic acids[1] (Kossel). The nucleins and nucleoproteides, compounds formed by the union of proteins and nucleic acids, are important constituents of all cell nuclei. For further details on the chemistry of these compounds the reader shoud consult Jones' Monograph on the Nucleic acids.

The view that the complex nucleic acids (polynucleotides) are made up of simpler fragments (mononucleotides) each of which possesses the composition of a simple nucleic acid, has been definitely proved by Levene and Jacobs. They have further shown that the mononucleotides are commonly composed of phosphoric acid, pentose (d-ribose) and a purine base united in the following fashion:

$$O = P\begin{matrix}\diagup OH \\ \diagdown OH\end{matrix}O \cdot C_5H_8O_3\text{—Purine, or pyrimidine, group}$$

The principal methods made use of in unravelling the mechanism of purine metabolism are the following:—

(1) The investigation of the action of various isolated tissues or tissue extracts, upon nucleic acids and on free purine derivatives.

(2) The administration of nucleic acids, nucleins, or nucleoproteides, to animals.

(3) The administration of free purine derivatives (adenine, hypoxanthine, guanine, xanthine and uric acid) to animals.

By utilizing the information gathered from each of these lines of inquiry, it has been possible to formulate a fairly satisfactory, although doubtless incomplete, picture of purine catabolism.

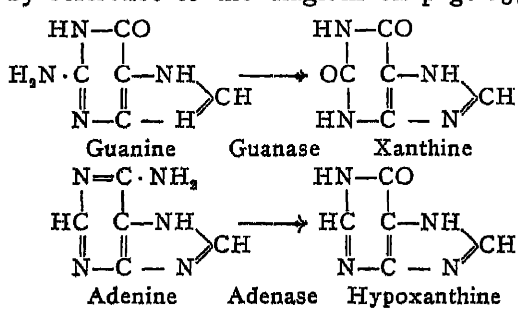
The nucleic acids, derived from the hydrolysis of nucleins and nucleoproteides, readily undergo further hydrolysis by means of

[1] For the most recent investigations upon the structure of nucleic acids, see papers by Levene and Jacobs, "Berichte," 1908—1911.

enzymes present in most animal tissues. These enzymes are collectively known as "nucleases" and act in a variety of ways. Thus, the nucleic acid may undergo complete hydrolysis yielding purine and pyrimidine bases, phosphoric acid and a carbohydrate. On the other hand, distinct nucleases may effect the liberation of phosphoric acid leaving the bases in combination with the sugar molecule (cf. Levene and Medegreceanu). Adenosine and guanosine are examples of substances formed in this reaction. They are collectively known as nucleosides and may be hydrolysed by acids, or enzymes found in various animal glands, yielding a purine base and a pentose (d-ribose). Their metabolism has been recently studied by Thannhauser and Bommes.

Another type of enzyme action involves the conversion of guanine, or adenine groups, while still attached to the sugar complex, into xanthine and hypoxanthine groups with liberation of ammonia.

Two other enzymes, not belonging to the class of nucleases, bring about the conversion of free guanine and adenine into xanthine and hypoxanthine. These are known as guanase and adenase respectively, and together with the last-mentioned enzymes belong to the class of so-called "desamidases" (Jones and Partridge, Jones and Winternitz). The relationships of these enzyme actions may be readily seen by reference to the diagram on page 135.



None of the above reactions are oxidations, but all of them are hydrolytic decompositions which are essential for the subsequent oxidation of the purine derivatives. *Eventually by the action of these several enzymes the purine groups guanine, xanthine, adenine and hypoxanthine present in the nucleic acids, or formed from them them by enzyme action, may be converted into free xanthine and hypoxanthine.*[1] (Cf. Diagram, p. 135.)

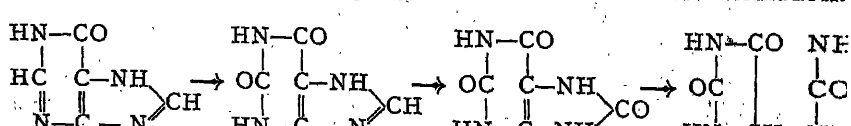
[1] For detailed information of the various steps involved in the biochemical hydrolysis of nucleic acids reference must be made to papers by Burian, Walter Jones, Levene, Salkowski, Schittenhelm and many others.

Horbaczewski showed in 1889 that uric acid was formed during the digestion of spleen pulp with blood in presence of oxygen, but that in the absence of blood xanthine and hypoxanthine, but no uric acid, were formed. Horbaczewski was under the impression that a certain amount of putrefaction was a necessary condition for uric acid production, but Spitzer and all subsequent observers showed this to be unnecessary. Kossel's fundamental investigations on the constitution of the nucleins led to the belief that the uric acid might be formed from purine bases derived from the nucleins of the spleen, and the probability of such a theory was greatly increased by the observation of Horbaczewski and a host of subsequent experimenters that nucleins, when fed to men, led to a large increase in the excretion of uric acid. Spitzer then demonstrated in 1899 the direct conversion of added xanthine into uric acid when digested with organ extracts in the presence of oxygen, and Wiener observed the similar formation of uric acid from hypoxanthine when digested with liver tissue.

In recent years the whole subject of the behaviour of various organs towards purine derivatives has been carefully re-investigated, especially by Burian, Jones, Schittenhelm and Wiechowski. It has been found that the purine bases undergo oxidation by means of enzymes which exhibit a very irregular distribution in various animal species. They are principally found in the glandular organs, especially liver, spleen and pancreas. It has been found possible to separate and to some extent purify these enzymes, so that their action upon added purine derivatives can be readily studied.

The principal steps in the oxidation of hypoxanthine and xanthine are as follows:—

1. Oxidation of hypoxanthine to xanthine.
2. Oxidation of xanthine to uric acid.
3. Oxidation of uric acid to allantoine and carbon dioxide.
4. Oxidation, or hydrolysis, of allantoine with urea formation.



Hypoxanthine Xanthine Uric acid Allantoine

The oxidations represented by reactions (1) and (2) are commonly ascribed to the action of a single enzyme, xanthine-oxidase. There

is some evidence which suggests the existence of separate enzymes for the oxidation of hypoxanthine and xanthine and by analogy with other enzymes the assumption appears probable (Wells). It is perhaps better to designate the enzymes concerned with the oxidation of hypoxanthine as hypoxanthine-oxidase reserving the name xanthine-oxidase for the enzyme taking part in the oxidation of xanthine to uric acid. These enzymes are found in the livers of most animals, including man. They are also found in many other glandular tissues of animals excepting man.

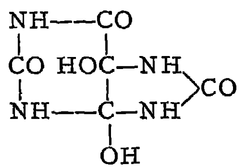
In effecting the oxidation of the purine bases to uric acid by means of tissue extracts the supply of oxygen profoundly affects the results. In the presence of but little oxygen an extract of dog's spleen oxidizes hypoxanthine to xanthine with practically no formation of uric acid, but when an abundance of oxygen is present uric acid is formed instead of xanthine.

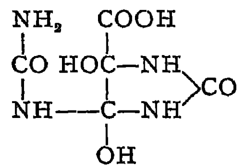
The oxidation of uric acid to allantoine (Reaction 3) is affected by an oxidizing enzyme known as "uricase", or as a "uricolytic enzyme". The action of this enzyme has been demonstrated in either the liver, or kidney, of all mammals thus far examined, with the exception of man, the chimpanzee and orang-otang. It is probably absent from all tissues of birds and reptiles, i.e. animals in which uric acid is the chief end-product of nitrogenous metabolism.

Wiechowski showed that on perfusing dog's liver or the kidney of the ox, with blood containing uric acid the latter was oxidized almost quantitatively to allantoine. Corresponding with this observation is the fact that ordinarily dogs urine contains much allantoine and very little uric acid. A curious exception to this rule has been observed by Benedict who found that dogs of the Dalmatian breed excrete considerable amounts of uric acid even when fed on a purine-free diet. An examination of the enzymes of dogs of this breed by Wells showed that the liver still possessed considerable power to effect the decomposition of uric acid. Neither the liver nor pancreas could oxidise xanthine to uric acid, but the former contained guanase and adenase.

The mechanism of the oxidation of uric acid to allantoine by means of potassium permanganate is complex, and apparently allantoine formation is preceded by the opening of both closed rings in the uric acid molecule. Behrend assumed that the formation of allantoine from uric acid was preceded by the formation of a so called uric acid glycol without actually being able to isolate the compound.

Later investigations by Biltz showed the feasibility of preparing such compounds synthetically and Behrend and Ziegler have now found that while the glycol is readily decomposed with liberation of ammonia by dilute alkali, neither allantoine nor uroxanic acid are produced. Uric acid glycol therefore cannot be regarded as an intermediate product of the oxidation of uric acid to allantoine by alkaline permanganate. Behrend and Ziegler now regard a substance with the following formula (II) as more probably a precursor of allantoine. This substance appears to be formed by condensation of alloxanic acid, $NH_2 \cdot CO \cdot NH \cdot CO \cdot CO \cdot COOH$, and urea in the presence of acetic anhydride but at once passes over into allantoine. The probability of its being a precursor of allantoine both in vitro and in vivo appears considerable.


I Uric Acid Glycol.


II

Nothing is known of any decomposition of allantoine by enzymes. In fact, the substance seems to be relatively resistant to changes in the animal body, and according to Wiechowski, is to be considered the normal end-product of the catabolism of purine bases by the carnivora.

Reference must be made at this point to an obscure phenomenon observed by Ascoli, Izar, Bezzola and Preti. These observers found that uric acid when added to oxygenated blood and perfused through a surviving liver practically completely disappears, but that on subsequently saturating the blood with carbon dioxide the uric acid reappears. Similar results were obtained with finely minced liver tissue under successively ærobic and anærobic conditions. This formation of uric acid is not observed to take place from added allantoine, parabanic acid, or glycine and urea. Dialuric acid and urea on the other hand, do lead to uric acid formation, but there is no convincing evidence that dialuric acid is formed from uric acid when perfused through a surviving liver with oxygenated blood. The phenomenon requires further investigation.

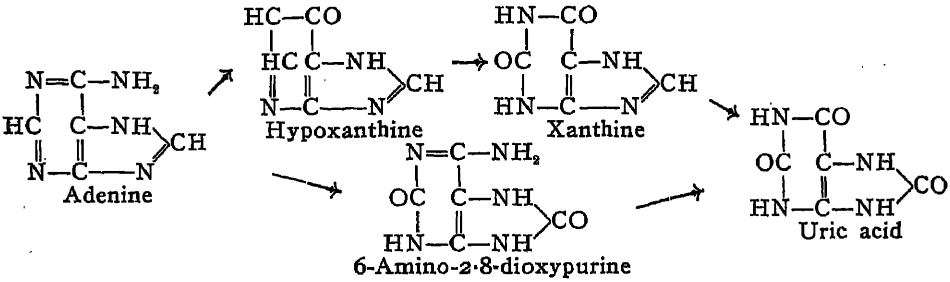
The fate in the animal body of the various purine derivative and their products of metabolism must now be considered. The results obtained by this line of investigation harmonize excellently with those derived from the study of the action of individual organ

although for a long while a satisfactory demonstration of the formation of uric acid from the xanthine bases was not obtained owing to the fact that the experiments were made on dogs, or other animals, promising a high capacity for uric acid destruction. Thus xanthine, guanine, and hypoxanthine may be fed to dogs without causing any marked increase in the excretion of uric acid, but when these same bases are administered to man an increased excretion of uric acid is readily observed.

Hypoxanthine, or xanthine, when administered to man leads to an increased output of uric acid corresponding to from 45 to 65 per cent. or even more of the base. The yield of uric acid from adenine and guanine has generally been found to be less than that from xanthine and hypoxanthine; the most reliable experiments give results varying from 10 to 40 per cent. according to conditions. (Minkowski, Burian and Schur, Krüger and Schmid, Mendel and Lyman, and others).

Although administration of purine bases to dogs commonly fails to produce an increased uric acid excretion, in several cases it has been possible to demonstrate the excretion of allantoine, which, as was previously mentioned, is produced in the liver of the dog by oxidation of uric acid. Minkowski, Cohn and Salkowski independently found a marked allantoine excretion follow feeding with thymus, or pancreas, both of these organs containing large quantities of purine bases in combination. Mendel and White observed similar results on injecting salts of nucleic acid, and recently Levene and Medegreceanu obtained much allantoine from inosine when given to dogs. Allantoine has also been obtained from the urine of dogs which had received hypoxanthine, guanine and adenine (Minkowski, Mendel and Lyman, Levene and Medegreceanu). There can be little doubt of the intermediate formation of the uric acid in the oxidation of xanthine bases to allantoine.

When adenine is administered to animals a peculiar form of nephritis is usually produced characterized by deposition in the kidneys of a sparingly soluble substance which has been identified by Nikolaier as 6-amino-2·8-dioxypurine. This substance would be expected to yield uric acid when acted upon by enzymes of the type of adenase. It would seem likely, therefore, that uric acid may be formed from adenine not only by conversion into hypoxanthine and subsequent oxidation to xanthine and uric acid, but also by first undergoing oxidation and subsequently parting with a NH_2 group to yield uric acid:—



The intravenous administration of guanine to rabbits was shown by Schittenhelm and Bendix to be followed by a large excretion of uric acid together with a smaller amount of xanthine, and Mendel and Lyman have obtained similar results. This observation harmonizes with the view that xanthine is a normal precursor of uric acid derived from the purine bases.

From what has been mentioned of the variations in the fate of purine bases when administered to different animal species, it would be anticipated that similar variations would be encountered with uric acid. This is found to be the case. Uric acid is oxidized in the human organism with much greater difficulty than is the case with other animals, including monkeys, dogs, cats, rabbits and pigs. When uric acid is administered to man more than half can usually be recovered unchanged from the urine, and in some cases as high a recovery as 99 per cent. has been recorded (Soetbeer and Ibrahim). The recent experiments of Wiechowski show an excretion of 60 to 90 per cent. of the uric acid administered subcutaneously. Burian and Schur's experiments led them to conclude that when uric acid was administered to man about 50 per cent. was excreted unchanged, with rabbits the proportion was about 15 per cent., while carnivorous animals excreted only about 4 per cent. Corresponding with these results are the observations that uric acid when administered to man fails to yield a marked increase in allantoine excretion, while in dogs uric acid readily yields allantoine (Salkowski, Mendel and White, Wiechowski, and others). Man's relative inability to effect the catabolism of uric acid may be referred to a more or less complete lack of the uricolytic enzyme present in the livers of most other animals, and actual experiments on the action of the human liver upon uric acid confirm this supposition. Wiechowski has, however, been able to isolate traces of allantoine from normal human urine which he considers to be derived from purine metabolism. It is possible therefore that the differences between various mammals with regard to uric acid decomposition is a quanti-

tative rather than a qualitative one. It is most probable, however, that the small amounts of allantoine found in human urine are derived from allantoine present in the food (Ackroyd).

In the past it has been generally assumed that urea was a quantitatively important product of the decomposition of uric acid, and hence probably of allantoine. It has been stated that uric acid on perfusion through a surviving liver yields urea (Ascoli, Subkow), but these experiments are hardly convincing when viewed in the light of Wiechowski's subsequent work on allantoine formation. According to Wiechowski the amount of allantoine excreted by dogs and rabbits after administration of uric acid is nearly sufficient to account for the whole of the uric acid undergoing oxidation. The amount of urea formed from uric acid under these conditions must therefore be small.

Levene and Medegreceanu, on the other hand, found comparatively ittle allantoine (15 per cent.) when sodium urate was given to dogs but much urea.

According to Wiechowski allantoine is the normal end-product of purine metabolism in rabbits and dogs. In agreement with this view it is found that allantoine is not very readily decomposed when given to animals. Wiechowski showed that when administered subcutaneously to man about 90 per cent. was excreted unchanged. Poduschka, on the other hand, found only 30 to 50 per cent. of allantoine excreted unchanged in the urine in cases of man, while with dogs he found almost quantitative excretion. It must be noted, however, that Poduschka failed to detect allantoine after feeding uric acid to dogs, and in addition all the methods for allantoine estimation prior to Wiechowski's work were very inaccurate. Recent experiments of Levene and Medegreceanu showed that when allantoine was given to dogs about one-third was excreted unchanged and two-thirds converted into urea. Luzatto's experiments also seemed to show a more ready decomposition. On administering three grams of allantoine to rabbits none could be recovered in the urine, but an increased excretion of oxalic acid was noted. This last result is of interest since it has frequently been suggested that oxalic acid might be an intermediate step in the oxidation in the body of uric acid. However, direct administration of uric acid does not lead to oxalic acid excretion (Luzatto and others). Outside the body allantoine is a relatively unstable substance undergoing slow decomposition in aqueous solution even at ordinary temperatures. This decomposition is much accelerated in an alkaline medium, and even Wiechowski is inclined to concede the possibility of oxalic acid formation from allantoine in the body under

certain conditions. Glycine is another substance which has been claimed to be derivable from uric acid (Wiener), but the evidence upon which this conclusion is based must be regarded as weak. At the same time it must be admitted that as allantoine is the di-ureide of glyoxylic acid,[1] and since the latter substance might readily yield either oxalic acid, or glycine,[2] the possibility of the formation of both of these substances from uric acid must be conceded.

In the diagram on p. 135, which is based upon that in Amberg and Jones's paper, are represented the principal paths of purine metabolism, showing how a typical nucleic acid, represented as a di-nucleotide containing adenine and guanine groupings is converted successively by hydrolysis and oxidation into xanthine, uric acid, allantoine and finally urea.

The oxidation of hypoxanthine to xanthine and subsequently to uric acid such as is believed to take place in the animal body has not yet been imitated *in vitro.* The oxidation of uric acid to allantoine, on the other hand, is readily effected by a variety of oxidizing agents such as potassium permanganate, lead peroxide, etc.

A word must be said of the possibility of the formation of purine bases by the reduction of uric acid. That purine bases may be synthesized in the young animal is well known. An inspection of careful urinary analyses following the administration of salts of uric acid shows in many cases a distinct rise in the purine nitrogen "other than uric acid". Although the evidence is admittedly slight, the idea of the reduction of uric acid to purine bases is by no means as incredible as might appear at first sight, for the reactions involved are not vastly different in character from those concerned in the observed reduction in the body of ketonic acids to hydroxy, or amino, acids. The possibility of such a reduction *in vitro* has been demonstrated recently by Sundwik though the reagents employed preclude any simple biochemical analogy. It was found that on heating uric acid at 200° with a mixture of glycerol and anhydrous oxalic acid that a thirty three per cent yield of xanthine could be obtained; while the reduction of xanthine to hypoxanthine was effected by heating with chloroform and sodium hydroxide.

[1] Salts of glyoxylic acid when administered to rabbits and dogs lead to a varying excretion of oxalic acid. Eppinger has stated that allantoine was formed by synthesis in the body from glyoxylic acid, but this result could not be confirmed by Dakin.

[2] Ammonia acts upon glyoxylic aid to give formylglycine (Erlenmeyer and Kunlin).

Some highly suggestive experiments by Ackroyd and Hopkins make it appear probable that histidine and arginine may function as sources of purines and a consideration of the structure of these amino acids is in harmony with this view. They found that one but not both of these amino acids was essential for maintenance and growth of young rats and that in their absence the normal excretion of allantoine was much diminished. Ackroyd had already shown that allantoine was the main end product of purine metabolism in the rat and that its amount in normal rat urine is about fifteen times as great as the combined excretion of purine bases and uric acid.

In connection with allantoine excretion reference may be made to a curious observation by E. Michaelis who found that the operation known as Claude Bernards puncture of the floor of the fourth ventricle in the brain of the dog is not only followed by glycosuria but by a transitory high excretion of allantoine. Michaelis is apparently inclined to the belief that a cerebral centre exists controlling purine metabolism in the liver.

Before concluding the consideration of the oxidation of the purine bases found in the animal body, reference may be made to the fate of the three methylxanthines of vegetable origin: theophylline, theobromine, and caffeine. All of these substances undergo a progressive demethylation, the order in which the methyl groups are removed varying in different animal species (Krüger and Schmidt). Whether demethylation is effected by oxidation, or hydrolysis, is unknown. Caffeine = 1, 3, 7-trimethylxanthine, when given to dogs chiefly yields 1, 3-dimethylxanthine (theophylline), 3-methylxanthine, 1, 7-dimethylxanthine and 3, 7-dimethylxanthine being formed in smaller amounts. Thus in dogs methyl groups (1) and (7) are most readily removed, while with rabbits it is found that methyl group (3) is most easily replaced, so that 1-methylxanthine, 7-methylxanthine and 1, 7-dimethylxanthine are excreted. Theobromine = 3, 7-dimethyl xanthine yields chiefly 3-methylxanthine when given to dogs and 7-methylxanthine when given to rabbits. Theophylline (1, 3-dimethylxanthine) yields 1, and 3-methylxanthine. Neither uric acid, methyluric acids nor methyl-allantoines have as yet been observed among the products of catabolism of methylated xanthines.

Little is known of the mode of decomposition of the pyrimidine bases present in various nucleic acids. Steudel has carried out feeding experiments with a number of these substances; he has not observed the synthesis of purine derivatives through combi-

nation with a urea grouping, nor identified any characteristic product of their catabolism.

Lewis has recently investigated the fate in the animal body of some hydantoins and parabanic acid, cyclic compounds structurally related to the purines. In each case it appeared that the substances were non-toxic, were not converted into urea and largely were excreted unchanged when fed to rabbits.

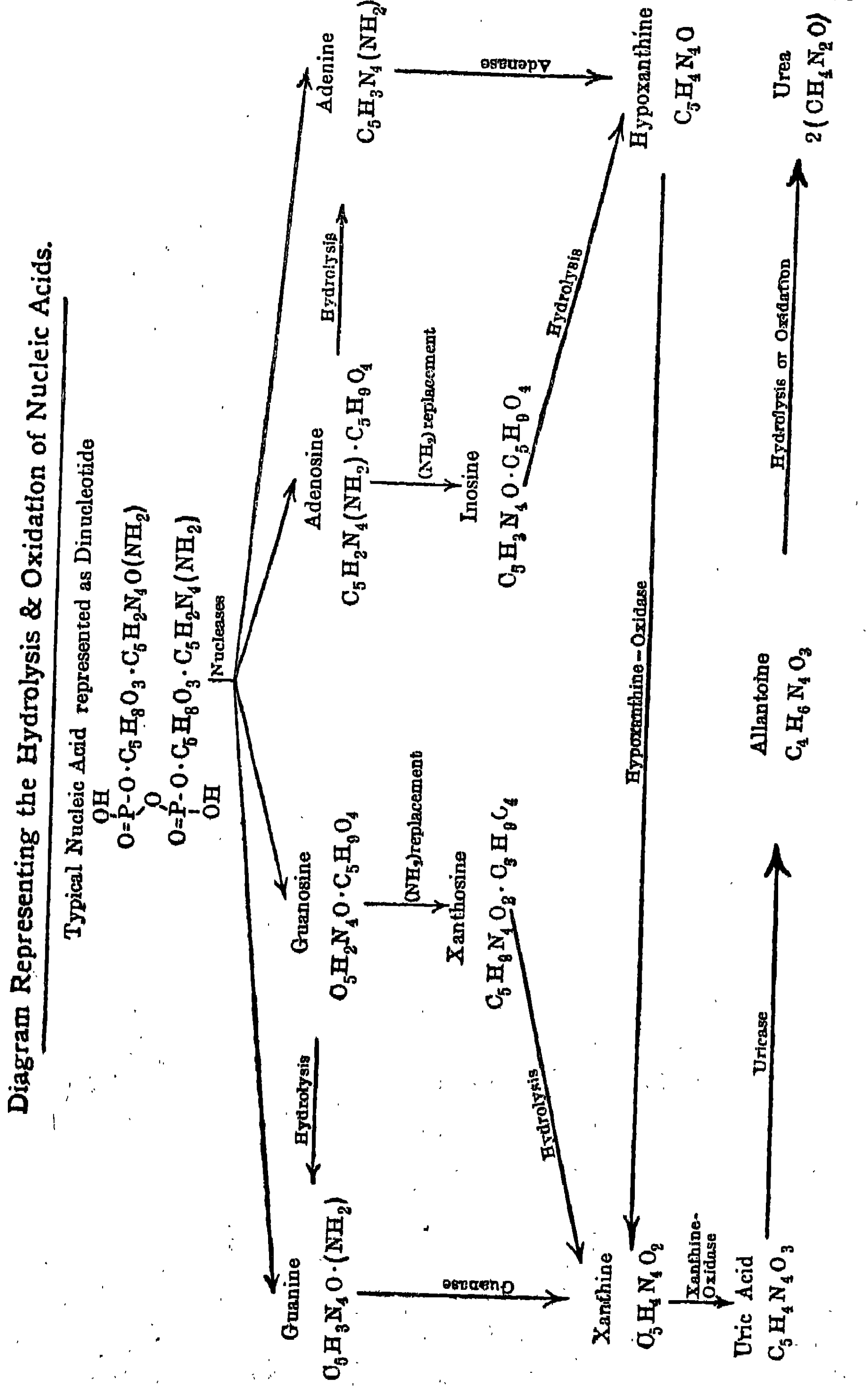


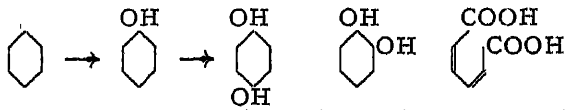
Diagram Representing the Hydrolysis & Oxidation of Nucleic Acids.
Typical Nucleic Acid represented as Dinucleotide
$O=P(OH)-O\cdot C_5H_8O_3\cdot C_5H_2N_4O(NH_2)$
O
$O=P(OH)-O\cdot C_5H_8O_3\cdot C_5H_2N_4(NH_2)$
Nucleases
Adenosine
$C_5H_2N_4(NH_2)\cdot C_5H_9O_4$
Hydrolysis
Adenine
$C_5H_3N_4(NH_2)$
Adenase
(NH_2) replacement
Inosine
$C_5H_3N_4O\cdot C_5H_9O_4$
Hydrolysis
Hypoxanthine
$C_5H_4N_4O$
Guanosine
$C_5H_2N_4O\cdot C_5H_9O_4$
(NH_2) replacement
Xanthosine
$C_5H_3N_4O_2\cdot C_5H_9O_4$
Hydrolysis
Hydrolysis
Guanine
$C_5H_3N_4O\cdot(NH_2)$
Guanase
Xanthine
$C_5H_4N_4O_2$
Hypoxanthine - Oxidase
Xanthine-Oxidase
Uric Acid
$C_5H_4N_4O_3$
Uricase
Allantoine
$C_4H_6N_4O_3$
Hydrolysis or Oxidation
Urea
$2(CH_4N_2O)$

CHAPTER VI.

HYDROCARBONS, PHENOLS, ALCOHOLS, ALDEHYDES, AMINES, INDOLE DERIVATIVES.

The Hydrocarbons. — The saturated hydrocarbons of the paraffin series up to and including the mixture of higher hydrocarbons known as vaseline, when administered to animals, appear to undergo some change in the animal body, but the nature of the reactions is unknown (Lassar, Lewin, Sobieranski). Experiments upon the fate of pure paraffins of known structure are entirely lacking.

The aromatic hydrocarbons, on the other hand, commonly undergo one, or both, of the following changes: (1) Oxidation with introduction of hydroxyl groups in the aromatic nucleus. (2) Oxidation of the side-chain with formation of acids. Thus, benzene when administered to animals is oxidized in part to phenol and catechol and to a lesser extent to quinol (Schultzen and Naunyn, Munk, Baumann and Herter, Nencki and Giacosa). Jaffé has found small quantities[1] of muconic acid in the urine of dogs and rabbits which had received considerable amounts of benzene, and this acid is clearly derived by the opening of the benzene ring by oxidation. It is possible, but not proved, that catechol is an intermediate step in the formation of muconic acid: —

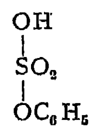


Benzene Phenol Quinol Catechol Muconic acid

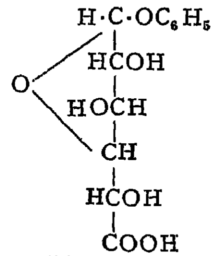
Earlier experiments by Jaffé appeared to show that muconic acid was very readily oxidised in the body, for he succeeded in recovering in the urine only about one per cent. of the acid fed. More recent experiments by Mori indicate that muconic acid is largely excreted unchanged when administered to rabbits thus making rather doubtful the view that benzene is principally oxidised in the animal body by way of muconic acid.

[1] About 0·3 per cent. of the benzene administered.

The phenols formed by the oxidation of aromatic hydrocarbons in the body are not excreted as such, but chiefly in combination with sulphuric acid in the form of salts of phenylsulphuric acid and to a lesser extent in combination with glucuronic acid. The constitution of phenylsulphuric acid and of phenylglucuronic acid has been definitely determined and confirmed by synthesis (Baumann, Salkowski and Neuberg).



Phenylsulphuric acid



Phenylglucuronic acid

The excretion of phenol when small amounts of benzene are administered to animals varies from about 15 to 30 per cent. of the theoretical amount. A close study of these variations under different conditions has been made by Nencki and Sieber in an attempt to utilize the reaction as a measure of the oxidative capacity of the body. A not inconsiderable part of the benzene is excreted unchanged by way of the lungs.

Apparently no formation of phenolic substances accompanies the oxidation of toluene, or ethylbenzene, or normal propylbenzene; all of these substances are oxidized to benzoic acid, which is excreted as hippuric acid (Schultzen and Naunyn, Nencki and Giacosa). But on the other hand, in addition to benzene, a large number of aromatic substances of varied types do undergo substitution of hydrogen atoms in the nucleus by hydroxyl groups to a more or less marked extent.[1] The imitation of this reaction *in vitro* usually can be effected only by a few oxidizing agents, including ozone, hydrogen peroxide, and by photochemical action.

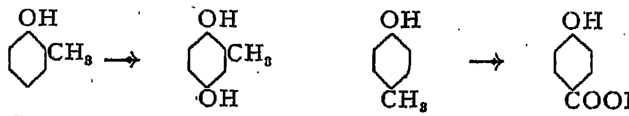
Thus phenol has been obtained from benzene by oxidation with hydrogen peroxide (Leeds); by ozone (Nencki and Giacosa); by air

[1] Including: chlorobenzene, bromobenzene, o- m- and p-dichlorobenzene, nitrobenzene, m-cymene, isopropylbenzene, isobutylbenzene, methylethyltoluene, mesitylene, diphenyl, p-bromodiphenyl, diphenylmethane, naphthalene, bromonaphthalene, phenol, o- and p-chlorphenol, p-cresol, anisol, phenetol, guaicol, thymol, and p-naphthol, aniline, dimethylaniline, formanilide, acetanilide, o-toluidine, p-acetotoluidide, phenylurethane, carbonyl-o-amidophenol, carbazol, acridine.

in the presence of palladium-hydrogen (Hoppe-Seyler), or of copper, or iron salts (Nencki and Sieber) — both of these latter reactions depending probably upon the formatiou of hydrogen peroxide—by the action of sunlight in the presence of caustic soda (Radziszewski) and also by the combined action of oxygen and aluminium chloride (Friedel and Crafts). Catechol and quinol may also be obtained by the oxidation of benzene and phenol with hydrogen peroxide in the presence of iron salts (Cross, Bevan, and Heiberg), and quinol is formed during the electrolytic oxidation of benzene in alcoholic solution (Gattermann and Friedrichs).

Phenols.—Phenol and paracresol are formed by the bacterial decomposition of tyrosine in the intestine. Small quantities of these substances are constantly being formed, and on absorption by the animal body they in part combine with sulphuric acid and to a lesser extent with glucuronic acid (Kulz), and are excreted in the urine. A considerable portion of the phenol, 50 per cent., or even more, undergoes oxidation with formation of quinol and catechol, which in turn combine with sulphuric acid, and to some extent with glucuronic acid, and are also excreted in the urine (Baumann and Preusse, Tauber, Schaffer). Catechol appears to be relatively resistant to further oxidation, but quinol appears to undergo oxidation more readily. Sufficiently accurate experiments to determine definitely the extent of the further oxidation of quinol and catechol in the body are at present lacking.

All of the isomeric cresols are in large measure excreted unchanged in combination with sulphuric acid. Ortho-cresol appears to give in addition a small amount of methylquinol, while p-cresol yields a small amount of p-hydroxybenzoic acid (Baumann and Herter). The latter substance is resistant to further oxidation in the animal body and is commonly found in small amounts in normal urine :—



o-Cresol Methylquinol p-Cresol p-Hydroxybenzoic acid

Alcohols.—In general the alcohols appear to undergo oxidation in the animal body with formation of the corresponding acids, but in many cases the latter substances undergo further oxidation and so escape detection. Administration of methyl alcohol to animals leads to a very definite excretion of formic acid (Pohl), and benzyl alcohol gives benzoic acid (hippuric acid). O. Neubauer showed that phenylethyl alcohol gave phenylacetic acid (phenaceturic acid), while Nencki found

that saligenin $C_6H_4OH \cdot CH_2OH$ was converted into salicylic acid. Many other cases of the oxidation of aromatic alcohols to the corresponding acids have been observed.

Ethyl alcohol when administered to animals is usually burned without the appearance of intermediate products of oxidation, although small amounts of unoxidised alcohol, both free and combined with glucuronic acid may be found in the urine. The volatile acids of the urine are not materially increased. It is probable, however, that acetic acid is formed by the oxidation of alcohol in the animal body and that it undergoes practically complete oxidation as fast as it is formed. The probability that acetaldehyde may be an oxidation product intermediate between alcohol and acetic acid follows from the experiments of N. Masuda who obtained substances with aldehydic properties on perfusion of livers supplied with alcohol containing blood. An increased acetoacetic acid production was noted in addition and this may be plausibly explained as originating from acetic acid which is now known to furnish acetoacetic acid when perfused through dog livers poor in glycogen.

The oxidation of alcohol to acetaldehyde in the body appears to be a partially reversible reaction, for Embden and Baldes have observed alcohol production on digesting acetaldehyde with liver tissue or on perfusion through a surviving liver. The alcohol probably originates as the result of the Cannizzaro reaction referred to in the next section dealing with aldehydes.

The oxidation of alcohol to acetic acid *via* acetaldehyde by animal tissues is apparently effected by enzyme action. The name alcoholoxidase' has been assigned to the enzyme by Battelli and Stern and a similar catalyst had previously been obtained by Buchner and Gaunt from the micro-organisms concerned with acetic fermentation.

Iso-amyl alcohol when added to blood used for perfusing a freshly excised liver, yields some acetoacetic acid, but possibly owing to its toxic action, the amount of acetoacetic acid is less than that obtained from iso-valeric aldehyde, or iso-valeric acid (F. Sachs). The latter substances are probably intermediate products in the reaction (cf. p. 55).

$$\begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!>\!CH \cdot CH_2 \cdot CH_2OH \rightarrow \begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!>\!CH \cdot CH_2 \cdot CHO \rightarrow$$

$$\begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!>\!CH \cdot CH_2 \cdot COOH \rightarrow CH_3 \cdot CO \cdot CH_2 \cdot COOH$$

Among the more complicated alcohols, cholesterol plays an important part in animal metabolism, though but little is known of the

chemical changes it undergoes in the body. Lifschütz has published many researches on the subject which indicate that in contact with blood and other tissues cholesterol may be oxidised to an oxy-cholesterol and other products of further oxidation. The characterisation of the oxidation products appears lacking in precision and further investigation is clearly necessary. Under similar conditions to those employed by Lifschütz for the oxidation of cholesterol it was found that phytosterol, the analogous alcohol derived from plants, is slowly and incompletely converted into a 'oxyphytosterol'.

Aldehydes.—It has long been known that aldehydes might be converted into acids in the animal body and the same reaction was observed to occur in various isolated animal tissues. Until recently the action was generally believed to be a simple oxidation due to oxidizing enymes collectively known as "aldehydases" (Schmiedeberg, Jaquet, Abelous and Biarnès, Jacoby, Spitzer, and others). It had, however, been noticed that free oxygen was not only unnecessary for the reaction, but under certain conditions was distinctly disadvantageous (Medwedew, Abelous and Aloy, Dony-Henault and Van Duuren). The explanation of this fact is found in the observations of Parnas and of Battelli and Stern, who have determined that the chief change which aldehydes undergo when brought in contact with animal tissues is not a direct oxidation at all, but is the so-called Cannizzaro reaction. Two molecules of the aldehyde undergo rearrangement in such a fashion that one molecule is *reduced* to the corresponding alcohol, while the second is *oxidized* to the corresponding acid. The acid and alcohol thus produced are either combined in the form of an ester, or free, according to the special conditions of the experiment. The Cannizzaro reaction is very commonly observed to occur *in vitro* when aldehydes are treated with alkalies:—

$$2R \cdot CHO + H_2O = R \cdot CH_2OH + R \cdot COOH$$

Parnas observed the Cannizzaro reaction to occur with considerable rapidity when the following aldehydes were digested with liver tissue in dilute sodium bicarbonate solution in the presence of oxygen: propionic, butyric, isobutyric, isovaleric, *n*-valeric, heptilic, benzoic, salicylic aldehydes and aldol. Batelli and Stern had already observed a similar decomposition with acetaldehyde, with formation of ethyl alcohol and acetic acid. Parnas suggests the name "aldehydemutase" for the enzyme concerned with the reaction.

In general it may be said that aldehydes may undergo rearrangement in the animal body with formation of the corresponding alco-

hols and acids, and that the alcohols may then undergo further oxidation with renewed formation of aldehyde and acid, so that the net result is a complete oxidation of the aldehyde to the corresponding acid. A large number of examples of this change have been observed in the case of aromatic as well as fatty aldehydes

The anomalous behaviour of furfurol in the animal body resulting in the synthesis of furfuracrylic acid has long been known. It was thought possible that urocanic acid (iminazolacrylic acid) might result from a similar change, but experiments by Barger and Dakin showed that no urocanic acid excretion followed the administration of glyoxalineformaldehyde to a dog but a small amount of glyoxaline carboxylic acid was detected. Friedmànn and Turk also failed to detect cinnamoylglycine after administering benzaldehyde, a result which the writer can confirm from independant experiments.

The reduction of many types of aldehydes to the corresponding alcohols through the action of fermenting yeast has been observed by Neuberg and his associates, but it is doubtful if a similar direct reduction occurs in the animal body. A rather interesting case of aldehyde decomposition involving the introduction of a hydroxyl group into a benzene nucleus has been observed by Tait. An old specimen of cinnamic aldehyde water which had become infected with bacterial growth was found to contain coumarin:

$$C_6H_5 \cdot CH=CH \cdot CHO \rightarrow C_6H_4(OH) \cdot CH=CH \cdot COOH \rightarrow C_6H_4\left\langle\begin{array}{l} O - CO \\ CH = CH \end{array}\right.$$

Amines.—Small amounts of a number of amines are constantly being formed by bacterial decomposition of amino acids in the intestine and subsequently are absorbed. Amines are also present in animal food; thus Krimberg found methylguanidine in fresh ox-muscle, while choline and other bases are found either free, or combined, in a variety of tissues.

The simple amines, methylamine, ethylamine and isoamylamine appear to undergo fairly complete decomposition in the animal body. Only small amounts appear unchanged in the urine (Salkowski, Schiffer, Erdmann). It has been stated that the corresponding alkyl ureas may also be excreted (Salkowski, Schmiedeberg), but these results lack adequate confirmation.

Formic acid appears to be an intermediate product of the oxidation of methylamine in the body, for Pohl observed a marked increase in formic acid excretion after administering 2 grms. of methylamine hydrochloride to a dog:

$$CH_3 \cdot NH_2 \rightarrow NH_3 + H \cdot COOH \rightarrow CO_2$$

No similar increase in volatile acids has been observed to follow the administration of the other aliphatic amines, possibly because the higher fatty acids readily undergo complete oxidation. But among the aromatic amines, benzylamine has been observed to give benzoic acid which is excreted as hippuric acid (Schmiedeberg, Mosso), and p-hydroxyphenylethylamine, a physiologically active base obtained by the decomposition of tyrosine, has been shown by Ewins and Laidlaw to give p-hydroxyphenylacetic acid. It is remarkable that the latter base appears capable of undergoing complete decomposition when perfused through a surviving heart.

Ewins and Laidlaw later experimented with indolethylamine, the base corresponding to tryptophan and found that on perfusion through the liver of the rabbit nearly half was converted into indole-acetic acid. More recently Guggenheim and Loeffler experimented with the same bases and were able to detect hydroxyphenylethyl alcohol and indole-ethyl alcohol as intermediate products of their oxidation. They suggest therefore that the catabolic path of typical amines may follow one or both of the lines indicated below:

$$R\cdot CH_2NH_2 \rightarrow R\cdot CH_2OH \rightarrow R\cdot CHO \rightarrow R\cdot COOH$$

$$R\cdot CH_2\cdot NH_2 \rightarrow R\cdot CHO \begin{array}{l} \nearrow R\cdot COOH \\ \searrow R\cdot CH_2OH \rightarrow R\cdot COOH \end{array}$$

In the first scheme the alcohol is supposed to be directly derived from the amine, while in the second it is formed from the aldehyde as the result of a Cannizzaro reaction. The second scheme is perhaps the more probable especially as Suto has shown that amines are readily oxidised to aldehydes by means of hydrogen peroxide and a trace of ferrous sulphate and it is known that the aldehydes, if formed, would undergo further change as indicated above.

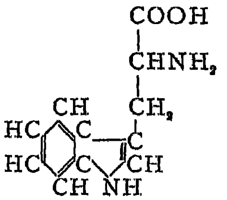
The formation of acetoacetic acid from isoamylamine when perfused through a surviving liver is probably preceded by formation of isovaleric acid which in turn readily yields acetoacetic (Sachs).

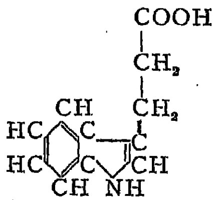
In general it will be noted that the behaviour of the simple primary amines in the body resembles that of the corresponding alcohols and acids.

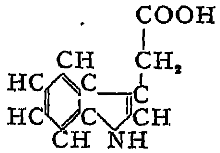
Both guanidine and methylguanidine, $NH_2\cdot C{=}NH\cdot NHCH_3$, appear to be very resistant against oxidation in the animal body, and when small doses are administered to animals the bases are excreted unchanged in the urine (Gergens and Baumann, Pommerenig).

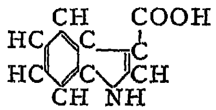
Concerning the fate of the other bases which may be formed in the animal organism there is an almost entire lack of definite information.

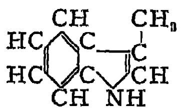
Indole Derivatives.—When tryptophan undergoes bacterial decomposition in the alimentary tract, or elsewhere, the following compounds may be formed: indole-β-propionic acid,[1] indole-β-acetic acid, scatole and indole (Hopkins and Cole, Salkowski, Nencki).


Tryptophan


Indole-β-propionic acid

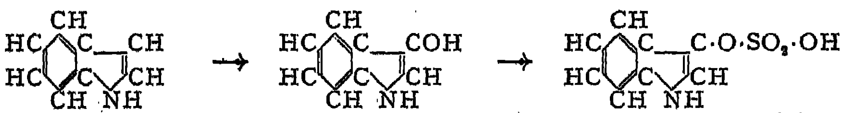

Indole-β-acetic acid


Indole-β-carboxylic acid


Scatole


Indole

The fate of indolepropionic acid in the body is unknown, but judging by analogy with other aromatic acids, e. g. phenylpropionic acid, one would expect indole-β-carboxylic acid to be formed by β-oxidation, and it may be noted that this latter substance appears to be actually present in urine under certain conditions. Indole-acetic acid, like phenylacetic acid, appears to be very resistant to change in the animal body and is a common constituent of most urines although the amount is not large (Salkowski, C. Herter). Indole when given to animals is oxidized to indoxyl, which is excreted in the urine in union with both sulphuric acid and glucuronic acid. Jaffé was the first to show that indole, which Nencki had found among the products of the putrefactive decomposition of proteins, was the mother substance of urinary indican, while Baumann and Brieger succeeded in isolating the potassium salt of the latter substance in a pure state and showed that, on hydrolysis with acids, it gave indoxyl and sulphuric acid. The urinary indican is usually known as indoxylsulphuric acid, owing to the fact that it yields indoxyl on hydrolysis, but indylsulphuric acid or indyl-hydrogen-sulphate is the more exact designation :—


Indole → Indoxyl → Indyl-hydrogen-sulphate

[1] Reduction of tryptophan to indole-β-propionic acid apparently is effected only by anærobic organisms.

Recently Neuberg and Schwenk isolated indoxylglucuronic acid from the urine of rabbits which had received relatively large amounts of both indole and sugar.

The oxidation of indole to indoxyl may be effected *in vitro* by means of hydrogen peroxide (Porcher). Much of the indoxyl is further oxidized with formation of isatin, indirubin and indigo blue. On warming an aqueous acetone solution of indole with hydrogen peroxide a precipitate of indigo blue is readily obtained.

When scatole is given to animals it has been stated that scatoxyl appears in the urine combined with sulphuric and glucuronic acids (Brieger). The constitution of urinary scatoxyl, if such a substance exists, which is doubtful, is unknown. The urines of animals which have received scatole develop a red colour on addition of concentrated hydrochloric acid (scatole red) and in addition yield indole on distillation (Jaffé). It is probable that this indole is derived from the slow decomposition of indole-β-carboxylic acid, derived from the oxidation of the methyl group in scatole, but Jaffé was unable to identify definitely the substance (cf. Blumenthal and E. Jacoby). It appears not improbable that indole-β-acetic acid and indole-β-carboxylic acid represent intermediate stages in the conversion of tryptophan into scatole and indole respectively, since micro-organisms are frequently capable of effecting the removal of carbon dioxide from carboxylic acids (cf. p. 99). This change, however, apparently does not take place so easily when the ready formed indole acids are subjected to the action of bacteria.

BIBLIOGRAPHY.

REFERENCES TO CHAPTER I.

BACH, A. *Peroxydasen als spezifisch wirkende Enzyme.* Ber., 1906, **39**, 2126.

BACH, A. *Du rôle des peroxydes dans les phénomènes d'oxydation lente.* Compt. rend., 1897, **124**, 951.

BACH, A. *Zur Kenntnis der Reduktionsfermente. I. Mitteilung. Ueber das Schardinger-Enzyme (Perhydridase).* Biochem. Zeitschr., 1911, **31**, 443.

BACH, A. *Zur Kenntnis der Reduktionsfermente. II. Mitteilung.* Biochem. Zeitschr., 1911, **33**, 282.

BACH, A. *Ueber den Mechanismus der Oxydationsvorgänge.* Ber., 1913, **46**, 3864.

BACH, A., AND R. CHODAT. *Untersuchungen über die Rolle der Peroxyde in der Chemie der lebenden Zelle. II. Ueber Peroxydbildung in der lebenden Zelle.* Ber., 1902, **35**, 2466.

BACH, A., AND R. CHODAT. *Untersuchungen über die Rolle der Peroxyde in der Chemie der lebenden Zelle. IV. Peroxydase.* Ber., 1903, **36**, 600.

BACH, A., AND R. CHODAT. *Untersuchungen über die Rolle der Peroxyde in der Chemie der lebenden Zelle. V. Zerlegung der sogenannten Oxydasen in Oxygenasen und Peroxydasen.* Ber., 1903, **36**, 606.

BACH, A., AND R. CHODAT. *Recherches sur les ferments oxydants.* Arch. d. Sciences Phys. et Nat., Geneva, 1904, **17**, 477.

BACH, A., AND R. CHODAT. *Untersuchungen über die Rolle der Peroxyde in der Chemie der lebenden Zelle. VIII. Ueber die Wirkungsweise der Peroxydasen.* Ber., 1904, **37**, 1342.

BACH, A., AND R. CHODAT. *Die Oxidationsvorgänge in der lebenden Zelle.* Biedermann's Zentralbl. f. agrik. Chem., 1908, **37**, 168.

BAEYER, A., AND V. VILLIGER. *Benzoylwasserstoff und die Oxydation des Benzaldehyds an der Luft.* Ber., 1900, **33**, 1569.

BATTELLI, F., AND L. STERN. *Die Oxydationsfermente.* Ergebnisse der Physiologie 1912, **12**, 96.

BAUMANN, E. *Zur Kenntnis des activen Sauerstoffs.* Zeitschrift f. physiol. Chem., 1881, **5**, 244.

BERTRAND, G. *Sur le pouvoir oxydant des sels manganeux et sur la constitution chimique des oxydases.* Compt. rend., 1897, **124**, 1355.

BODLÄNDER, G. *Ueber langsame Verbrennung.* Ahren's Vorträge 1889, 385 (Enke, Stuttgart).

BONE, W. A., AND J. DRUGMAN. *The explosive combustion of hydrocarbons.* Trans. Chem. Soc., 1906, **89**, 660. *And many other papers in* Trans. Chem. Soc.

BREDIG, G. *Ueber den Mechanismus der Oxydationsvorgänge.* Ber., 1914, **47**, 546.

CIAMICIAN, G., AND P. SILBER. *Chemische Lichtwirkungen.* XVIII. Ber., 1910, **43**, 1536. *Also many earlier papers in the* Berichte.

CLAUSIUS. *Ueber den Unterschied zwischen activem und gewöhnlichem Sauerstoff.* Poggendorff's Annalen, 1884, **121**, 250.

ENGLER, C., AND W. WILD. *Ueber die sogenannte Aktivierung des Sauerstoffs und über Superoxydbildung.* Ber., 1897, **30**, 1669.

ENGLER, C., AND R. O. HERZOG. *Zur chemischen Erkenntnis biologischer Reaktionen.* Zeitschr. physiol. Chem. 1909, **59**, 327.

ENGLER, C., AND J. WEISSBERG. *Kritische Studien über die Vorgänge der Autoxydation.* Vieweg und Sohn, Braunschweig, 1904.

FENTON, H. J. H. *Oxidation of tartaric acid in presence of iron.* Trans. Chem. Soc., 1894, **65**, 899.

FISCHER, F., AND W. SCHNEIDER. *Einiges über die bei der Oxydation des Paraffins entstehenden Fettsäuren.* Ber., 1920, **53**, 922.

GRÜN, A. *Die Oxydation von Paraffin.* Ber., 1920, **53**, 987.

HOPKINS, F. G. *On an Autoxidisable Constituent of the Cell.* Biochem. Journ., 1921, **15**, 286.

HOPPE-SEYLER, B. *Ueber Gährungsprozesse.* Zeitschr. f. physiol. Chem., 1878, **2**, 1.

KASTLE, J. H, *The oxidases and other oxygen-catalysts concerned in biochemical oxidations.* Bulletin No. 59, Hygienic Laboratory, Washington Public Health and Marine Hospital Service of the United States.

KELBER, C. *Die Oxydation von Kohlenwasserstoffen mit Sauerstoff.* Ber., 1920, **53**, 66.

MUMMERCY, C. S. *Studies on oxidation* II. Journ. Soc. Chem. Ind., 1913, **32**, 889.

RADZISZEWSKI, B. *Ueber die Phosphorescenz organischer und organisierter Körper.* Annalen, 1881, **203**, 305.

REY-PAILHADE, J. *Philothion in the lens of Eyes.* C. r. Acad. des Science, 1915, **160**, 37.

RÖHRMANN, F., AND T. SHMAMINE. *Ueber komplexe Verbindungen von Ferrosalzen, Wasserstoffsuperoxyd und Eiweissstoffen.* Biochem. Zeitschr., 1912, **42**, 235.

SCHARDINGER, F. *Ueber das Verhalten der Kuhmilch zu Methylenblau bezw. Formalinmethylenblau.* Chem. Zeit., 1904, **28**, 704.

STOERMER, R. *Die Oxgdations- und Reduktionsmethode der organischen Chemie.* Leipsic, 1909. G. Thieme. Sonderabdruck aus Weyl's Handbuch, *Die Methoden der organischen Chemie.*

TRAUBE, M. *Zur Theorie der Gährungs- und Verwesungserscheinungen, wie der Fermentwirkungen überhaupt.* Poggendorff's Annalen, 1858, **103**, 331.

TRAUBE, M. *Die chemische Theorie der Fermentwirkungen und der Chemismus der Respiration.* Ber., 1878, **11**, 1984.

TRAUBE, M. *Ueber Activirung des Sauerstoffs.* I-IV. Ber., 1881, **15**, 659, 2421, 2434; 1883, **16**, 123.

TRAUGE, M. *Gesammelte Abhandlungen.* Mayer und Müller, Berlin, 1899.

VAN'T HOFF, J. *Ueber den Vorgang der langsamen Oxydation.* Chem. Zeitung, 1896, **20**, 807.

WIELAND, H. *Ueber Hydrierung und Dehydrierung.* Ber., 1912, **45**, 484.

WIELAND, H. *Studien über die Oxydationsvorgänge.* Ber., 1912, **45**, 2606; 1913, **46**, 3327, 3864.

WOLFF, J. *Sur quelques sels minéraux qui jouent le rôle de peroxydases.* Compt. rend., 1908, **146**, 142, 781, 1217.

WOLFF, J. *Contributions à la connaissance de divers phénomènes oxydasiques naturels de artificiels.* Laval, Barnéoud, 1910.

REFERENCES TO CHAPTER II.

THE FATTY ACIDS.

AUTENRIETH, W., AND H. BARTH. *Ueber Vorkommen und Bestimmung der Oxalsäure im Harn.* Zeitschr. f. physiol. Dhem., 1902, **35**, 327.

BAER, J., AND L. BLUM. *Ueber den Abbau von Fettsäuren beim Diabetes mellitus.* I. Mitteilung. Arch. f. exper. Path. u. Pharm., 1906, **55**, 89.

BAER, J., AND L. BLUM. *Ueber den Abbau von Fettsäuren beim Diabetes mellitus.* II. Mitteilung. Arch. f. exper. Path. u. Pharm., 1906, **56**, 92.

BAER, J., AND L. BLUM. *Ueber den Abbau von Fettsäuren beim Diabetes mellitus.* III. Mitteilung. Arch. f. exper. Path. u. Pharm., 1908, **59**, 321.

BAER, J., AND L. BLUM. *Ueber den Abbau von Fettsäuren beim Diabetes mellitus.* IV. Mitteilung. Arch. f. exper. Path. u. Pharm. 1910, **62**, 129

BAER, J., AND L. BLUM. *Ueber die Einwirkung chemischer Substanzen auf die Zuckerausscheidung und die Acidose.* Arch. f. exper. Path. u. Pharm., 1911, **65**, 1.

BAKHOVEN, L. L. *Ueber die Ausscheidung der Oxalsäure beim Menschen.* Maly's Jahrb. f. Tierchemie, 1902, **32**, 362.

BATTELLI, F., AND L. STERN. *Die Oxydation der Bernsteinsäure durch Tiergewebe.* Biochem. Zeitschr., 1911, **30**, 172.

BATTELLI, F., AND L. STERN. *Die Oxydation der Zitronen-, Äpfel- und Fumarsäure durch Tiergewebe.* Biochem. Zeitschr., 1911, **31**, 478.

BERGELL, P. *Das Verhalten der Salze organischer Säuren im Organismus.* Verhandl. d. Kongr. f. innere Med., 1907, **24**, 236.

BLUM, L. *Ueber den Abbau von Fettsäuren im Organismus und über die gegenseitigen Beziehungen der Azetonkörper.* Münch. med. Woch., 1910, **57**, 683.

BLUM, L., AND M. KOPPEL. *Ueber die Bildung von Methyl-propyl-keton aus Diaethylessigsäure im tierischen Organismus.* Ber., 1911, **44**, 3576.

BLUM, L., AND NAKANO. *The Influence of Hyperglycaemia on the Formation of Acetone Bodies.* Compt. r. Soc. de Biologie, 1919, **82**, 1435.

BLUM, L., AND P. WORINGER. *Decomposition of Propionic Acid in the Animal Body.* Bull. Soc. chim. Biol. 1920, **2**, 88.

BODCKER, E. *Ueber die Oxydationsprodukte der Sebacinsäure.* J. prakt. Chem., 1912, [2], **85**, 221.

BOUGAULT, J., AND C. CHARAUX. *Laetarinic Acid.* Compt. rend., 1911, **153**, 880.

BREDET, J. *Einwirkung von Salpetersäure auf Fettsäuren, welche die Isopropylgruppe enthalten.* Ber., 1881, **14**, 1780.

BRION, A. *Ueber die Oxydation der stereoisomeren Weinsäuren im tierischen Organismus.* Zeitschr. f. physiol. Chem., 1898, **25**, 283.

BUCHHEIM, R. *Ueber den Uebergang einiger organischen Säuren in den Harn.* Archiv f. physiol. Heilk., New Series, 1857, **1**, 122.

CAHEN, E., AND W. H. HURTLEY. *The oxidation of the Alkali Butyrates with Hydrogen Peroxide with the Production of Succinic Acid.* Biochem. Journ., 1917, **11**, 164.

DAKIN, H. D. *Experiments bearing upon the mode of oxidation of simple aliphatic substances in the animal organism.* Journ. Biol. Chem., 1907, **3**, 57.

DAKIN, H. D. *The oxidation of butyric acid by means of hydrogen peroxide with formation of acetone, aldehydes, and other products.* Journ. Biol. Chem., 1908, **4**, 77.

DAKIN, H. D. *The oxidation of ammonium salts of hydroxy fatty acids with hydrogen peroxide.* Journ. Biol. Chem., 1908, **4**, 91.

DAKIN, H. D. *A synthesis of certain naturally occurring aliphatic ketones with a suggestion of a possible mode of formation of these substances in the organism.* Journ. Biol. Chem., 1908, **4**, 221.

DAKIN, H. D. *A comparative study of the oxidation of the ammonium salts of saturated fatty acids with hydrogen peroxide.* Journ. Biol. Chem., 1908, **4**, 227.

DAKIN, H. D. *Comparative studies of the mode of oxidation of phenyl derivatives of fatty acids by the animal organism and by hydrogen peroxide.* Journ. Biol. Chem., 1908, **4**, 419.

DAKIN, H. D. *Einige Bemerkungen zu der Mitteilung von Friedmann. "Zur Kenntnis des Abbaues der Karbonsäuren im Tierkörper".* Beitr. z. chem. Phys. u. Path., 1908, **11**, 405.

DAKIN, H. D. *Further studies of the mode of oxidation of phenyl derivatives of fatty acids in the animal organism (phenylbutyric acid, phenyl-β-oxybutyric acid, phenylacetone, phenylisocrotonic acid, phenyl-β-γ-dioxybutyric acid).* Journ. Biol. Chem., 1908, **5**, 173.

DAKIN, H. D. *Further studies of the mode of oxidation of phenyl derivatives of fatty acids in the animal organism.* III. Journ. Biol. Chem., 1908, **5**, 303.

DAKIN, H. D. *The mode of oxidation in the animal organism of phenyl derivatives of fatty acids.* Part IV. Journ. Biol. Chem., 1909, **6**, 203.

DAKIN, H. D. *The mode of oxidation in the animal organism of phenyl derivatives of fatty acids.* Part V. *Studies on the fate of phenylvaleric acid and its derivatives.* Journ. Biol. Chem., 1909, **6**, 221.

DAKIN, H. D, *The fate of benzoylacetic acid in the animal body.* Journ. Biol. Chem., 1911, **9**, 123.

DAKIN, H. D. *The formation in the animal body of β-hydroxybutyric acid by the reduction of acetoacetic acid.* Journ. Biol. Chem., 1910, **8**, 97.

DAKIN, H. D. *A general reaction for the conversion of saturated fatty acids* ($R\cdot CH_2\cdot CH_2\cdot COOH$) *into ketones* ($R\cdot CO\cdot CH_3$). Amer. Chem. Journ., 1910, **44**, 41.

DAKIN, H. D., N. W. JANNEY, AND A. J. WAKEMAN. *Studies on the conditions affecting the formation and Excretion of Formic Acid.* Journ. Biol. Chem., 1913, **14**, 341.

DAKIN, H. D., AND A. J. WAKEMAN. *On the decomposition of β-hydroxybutyric acid and acetoacetic acid by enzymes of the liver.* Journ. Biol. Chem., 1909, **6**, 373.

DAKIN, H. D., AND A. J WAKEMAN. *On the decomposition of acetoacetic acid by the enzymes of the liver.* Part II. Journ. Biol. Chem., 1910, **8**, 105.

DAKIN, H. D., AND A. J. WAKEMAN. *Formic Acid as an intermediary substance in the catabolism of fatty acids and other substances (Preliminary Note).* Journ. Biol. Chem., 1911, **9**, 329.

EINBECK, H. *Ueber das Vorkommen von Bernsteinsäure im Fleischextrakt und im frischen Fleische.* Zeitschr. physiol. Chem., 1913, **87**, 145.

EINBECK, H. *Ueber das Vorkommen von Fumarsäure im frischen Fleische.* Zeitschr. f. physiol. Chem., 1914, **90**, 301.

EINBECK, H. *Ueber quantitative Versuche mit dem Succinoxydon von Battelli und Stern.* Biochem. Zeitschr., 1919, **95**, 296.

EMBDEN, G. *Beitrag zur Lehre von der Acetonurie.* Verhandl. d. Kongr. f. innere Med., 1907, **24**, 252.

EMBDEN, G., AND H. ENGEL. *Ueber Acetessigsäurebildung in der Leber.* Beitr. z. chem. Physiol. u. Path., 1908, **11**, 323.

EMBDEN, G., AND F. KALBERLAH. *Ueber Acetonbildung in der Leber.* I. Beitr. z. chem. Physiol. u. Path., 1906, **8**, 121.

EMBDEN, G., AND L. LATTES. *Ueber die Acetessigsäurebildung in der Leber des diabetischen Hundes.* Beitr. z. chem. Physiol. u. Path., 1908, **11**, 327.

EMBDEN, G., AND A. LOEB. *Ueber die Acetessigsäurebildung aus Essigsäure.* Zeitschr. physiol. Chem. 1913, **88**, 246.

EMBDEN, G., AND MARX. *Ueber Acetonbildung in der Leber.* III. Mitteilung. Beitr. z. chem. Physiol. u. Path., 1908, **11**, 318.

EMBDEN, G., AND L. MICHAUD. *Ueber den Abbau der Acetessigsäure im Tierkörper.* Mitteilung I. Beitr. z. chem. Physiol. u. Path., 1908, **11**, 332.

EMBDEN, G., AND L. MICHAUD. *Ueber den Abbau der Acetessigsäure im Tierkörper.* Mitteilung II. Biochem. Zeitschr., 1908, **13**, 262.

EMBDEN, G., H. SALOMON, AND FR. SCHMIDT. *Ueber Acetonbildung in der Leber.* II. Mitteilung. *Quellen des Acetons.* Beitr. z. chem. Physiol. u. Path., 1906, **8**, 129.

EMBDEN, G., AND J. WIRTH. *Ueber Hemmung der Acetessigsäure in der Leber.* Biochem. Zeitschr., 1910, **27**, 1.

ERDMANN UND MARCHAND. *Umwandlung der Zimmtsäure in Hippursäure.* Annalen, 1842, **44**, 344.

FAUST, E. *Ueber die Ursache der Gewöhnung an Morphin.* Achiv. f. exper. Path. u. Pharm., 1900, **44**, 236.

FITTIG, R. *Ueber Umlagerungen bei den ungesättigten Säuren.* I. Annalen, 1894, **283**, 47.

FLEIG, M. C. *Étude physiologique de quelques composés formiques.* Archives Internationales de Pharmacodynamie et de Thérapie, 1907, **17**, 147.

FLEIG, M. C. *L'élimination urinaire des formiates.* Arch. inter. Pharmacodynamie et Thérapie, 1908, **18**, 89.

FOLIN, O. *On the separate determination of acetone and diacetic acid in diabetic urines.* Journ. Biol. Chem., 1907, **3**, 177.

FRIEDMANN, E. *Zur Kenntnis des Abbaues der Carbonsäuren im Tierkörper.* Mitteilung V. *Ueber eine Synthese der Acetessigsäure bei der Leberdurchblutung.* Beitr. z. chem. Physiol. u. Path., 1908, **11**, 202.

FRIEDMANN, E. *Zur Kenntnis des Abbaues der Carbonsäuren im Tierkörper.* Mitteilung VII. *Ueber die Bildung von Acetessigsäure aus Isovaleriansäure bei der Leberdurchblutung.* Beitr. z. chem. Physiol. u. Path., 1908, **11**, 365.

FRIEDMANN, E. *Zur Kenntnis des Abbaues der Carbonsäuren im Tierkörper.* Mitteilung VIII. *Ueber das Verhalten der α-β-ungesättigten Säuren bei der Leberdurchblutung.* Beitr. z. chem. Physiol. u. Path., 1908, **11**, 371.

FRIEDMANN, E. *Ueber den Abbau der Fettsäuren im Tierkörper.* Medizinische Klinik, 1909, No. 36, 37 (Vortrag, gehalten in der Physiologischen Gesellschaft zu Berlin, 2. Juli, 1909).

FRIEDMANN, E. *Zur Kenntnis des Abbaues der Karbonsäuren im Tierkörper.* RI. *Verhalten der Benzoylessigsäure im Tierkörper.* Biochem. Zeitschr. 1910, **27**, 119.

FRIEDMANN, E. *Neuere Versuche über den physiologischen Abbau der Fettsäuren.* Medizinische Klinik, 1911, No. 28 (Vortrag, gehalten in der Physiologischen Gesellschaft zu Berlin, 5. Mai, 1911).

FRIEDMANN, E. *Zur Kenntnis des Abbaues der Karbonsäuren im Tierkörper.* XIV. Mitteilung. *Ueber Dehydrierung im Tierkörper.* Biochem. Zeitschr., 1911, **35**, 49.

FRIEDMANN, E. *Verhalten der Furfuracrylsäure und der Furoylessigsäure im Tierkörper.* Biochem. Zeitschr., 1911, **35**, 40.

FRIEDMANN, E. *Ueber die Bildung der Acetessigsäure aus Essigsäure bei der Leberdurchblutung.* Biochem. Zeitschr. 1913. **55**, 436.

FRIEDMANN, E. *Weitere Versuche über die Bildung von l-β-Oxybuttersäure aus Crotonsäure durch Leberbrei.* Biochem. Zeitschr., 1914, **61**, 281.

FRIEDMANN, E., AND C. MAASE. *Zur Kenntnis des Abbaues der Karbonsäuren im Tierkörper.* X. *Verhalten der -α-β-Dioxysäuren im Tierkörper.* Biochem. Zeitschr., 1910, **27**, 113.

FRIEDMANN, E., AND C. MAASE. *Zur Kenntnis des Abbaues der Karbonsäuren im Tierkörper.* XII. *Ueber eine neue Bildungsweise der β-oxybuttersäure im Tierkörper.* Biochem. Zeitschr., 1910, **27**, 474.

FRIEDMANN, E., AND C. MASSE. *Ueberführung von Crotonsäure in l-β-Oxybuttersäure durch Leberbrei.* Biochem. Zeitschr. 1913, **55**, 450.

GAGLIO, G. *Ueber die Unveränderlichkeit des Kohlenoxydes und der Oxalsäure im tierischen Organismus.* Arch. f. exper. Path. u. Pharm., 1887, **22**, 235.

GEELMUYDEN, H. C. *Ueber Aceton als Stoffwechselprodukt.* Zeitschr. f. physiol. Chem., 1897, **23**, 431.

GEELMUYDEN, H. C. *Ueber das Verhalten der Acetonkörper im intermediären Stoffwechsel.* Zeitschr. f. physiol. Chem., 1911, **73**, 176.

GREHANT ET QUINQUAD. *Que devienent les formiates introduits dans l'organisme.* Compt. rend., 1887, **104**, 437.

HERMANNS, L. *Ueber den Abbau der β-Ketonsäuren im tierischen Organismus.* Zeitschr. f. physiol. Chem., 1913, **85**, 233.

HILDEBRANDT, H. *Ueber eine experimentelle Stoffwechselabnormität.* Zeitschr. f. physiol. Chem., 1902, **35**, 141.

HIRSCHFELD, F. *Beobachtungen über die Acetonurie und das Coma diabeticum.* Zeitschr. f. klin Med.. 1895, **28**, 176.

HOPKINS, F. G., AND S. W. COLE. *On the proteid reaction of Adamkiewicz with contributions to the chemistry of glyoxylic acid.* Proc. Roy. Soc., 1901, **68**, 21.

KENNAWAY, E. L. *The relative amounts of β-hydroxybutyric acid and acetoacetic acid excreted in acetonuria.* Biochem. Journ., 1914, **8**, 355.

KNOOP, F. *Der Abbau aromatischer Fettsäuren im Tierkörper.* Beiträge z. chem. Physiol. u. Path., 1904, **6**, 150.

KNOOP, F. *Der Abbau aromatischer Fettsäuren im Tierkörper.* Monograph. Ernst Kuttruff, Freiburg (Baden).

KNOOP, F. *Zur Oxydation von Fettsäuren.* Beitr. z. chem. Physiol. u. Path., 1908, **11**, 411.

KOEHNE, F. *Ueber das Verhalten einiger Säureimide im tierischen Organismus.* Inaug. Dissertation, Rostock, 1904.

LAGERMARK, L. VON. *Ueber die Verbreitung der Ketoreduktase in den Geweben.* Biochem. Zeitschr., 1913, **55**, 448.

LEATHES, J. B., AND L. MEYER WEDELL. *Desaturation of fatty acids in the Liver.* Journ. Physiol., 1909, **38**, Proc. XXXVIII-XL.

LOEB, A. *Ueber das Verhalten der Essigsäure bei künstlicher Durchblutung der Leber.* Biochem. Zeitschr., 1912, **47**, 118.

LOEB, A. *Ueber die Acetessigsäurebildung aus Glykolsäure in der Leber.* Zeitschr. f. physiol. Chem., 1915, **93**, 270.

LOMMEL, F. *Ueber die Herkunft der Oxalsäure im Harn.* Deutsches Arch. f. klin. Med., 1899, **63**, 599.

LUZZATTO, A. M. *Zur Physiologie der Oxalsäure und Oxalursäure im Harn.* Zeitschr. f. physiol. Chem., 1903, **37**, 225.

LUZZATTO, R. *Ueber den physiologischen Abbau der Säuren der Propanreihe.* Beitr. z. chem. Physiol. u. Path., 1906, **7**, 456.

MARFORI, P. *Ueber die Umwandlungen einiger Säuren der Oxalsäurereihe im menschlichen Organismus.* Maly's Jahresber. f. Thierchemie, 1890, **20**, 70.

MARFORI, P. *Ueber die Veränderungen einiger Säuren der Oxalsäurereihe durch den Organismus. Malonsäure, Bernstein- und Glutarsäure.* Maly's Jahresber. f. Thierchemie, 1896, **26**, 74.

MARRIOTT, W. K. *The metabolic relationships of the Acetone bodies.* Journ. Biol. Chem. 1914, **18**, 241.

MCKENZIE, A. *The resolution of β-hydroxybutyric acid into its optically active components.* Trans. Chem. Soc., 1902, **81**, 1402.

MAGNUS-LEVY, A. *Die Oxybuttersäure und ihre Beziehungen zum Coma diabeticum.* Arch. f. exper. Path. u. Pharm., 1899, **42**, 149.

MALLEVRE, A. *Der Einfluß der als Gärungsprodukte der Zellulose gebildeten Essigsäure auf den Gaswechsel.* Pflüger's Archiv, 1891, **49**, 460.

MEYER, R. *Untersuchungen über Hydroxylierung durch direkte Oxydation.* Annalen, 1883, **219**, 240.

MINKOWSKI, O. *Untersuchungen über den Diabetes Mellitus nach Extirpation des Pankreas.* Arch. f. exper. Path. u. Pharm., 1893, 31, 85.

MOCHIZUKI, J. *Das Verhalten der Glykolsäure und der Glyoxylsäure bei der Leberdurchblutung.* Biochem. Zeitschr., 1913, **55**, 443.

MOCHIZUKI, J. *Ueber Acetessigsäurebildung bei der Leberdurchblutung glykogenreicher Tiere.* Biochem. Zeitschr., 1913, **55**, 446.

NENCKI, M. *Die Oxydation von Acetophenon im Tierkörper.* Journ. f. prakt. Chem., 1878, **18**, 288.

NEUBAUER, O. *Ein Beitrag zur Kenntnis der diabetischen Acidose.* Verhandlg. d. Kongr. f. inn. Med., 1910, 566.

NEUBERG, C., AND S. SANEYOSHI. *Ueber das Verhalten der stereoisomeren Weinsäuren im Organismus des Hundes.* Biochem. Zeitschr., 1911, **36**, 32.

OHTA, K. *Ueber das Verhalten der Äpfelsäure im Tierkörper.* Biochem. Zeitschr. 1912, **44**, 481.

POHL, J. *Ueber den oxydativen Abbau der Fettkörper im tierischen Organismus.* Arch. f. exper. Path. u. Pharm , 1895, **37**, 413.

PRIBRAM, B. O. *Die Verwertung der β-oxybuttersäure und die Bedeutung der Acetessigsäure in der normalen und diabetischen Leber.* Zeitschr. f. exper. Path. u. Ther., 1912, **10**, 284.

RAPER, H. S. *Note on the occurrence of an Octoic (caprylic) acid in butyric fermentation; and the occurrence of hexoic acid in butter fat.* Journ. Physiol., 1907, **35**, Proc. XXIV-XXXVI.

RAPER, H. S. *Condensation of acetaldehyde and its relation to the biochemical synthesis of fatty acids.* Trans. Chem. Soc., 1907, **91**, 1831.

RAPER, H. S. *The mode of oxidation of certain fatty acids with branched chains.* Biochem. Journ., 1914, **8**, 320.

RINGER, A. I. *The quantitative conversion of propionic acid into glucose.* Journ. Biol. Chem., 1912, **12**, 511.

RINGER, A. I. *The formation of glucose from valerianic and heptylic acid.* Journ. Biol. Chem., 1913, **14**, 43.

RINGER, A. I., E. M. FRANKEL, AND L. JONAS. *The fate of isobutyric, isovalerianic and isocaproic acid in the diabetic organism.* Journ. Biol. Chem., 1913, **14**, 525.

RINGER, A. I., E. M. FRANKEL, AND L. JONAS. *The fate of succinic, malic and malonic acid in the diabetic organism.* Journ. Biol. Chem., 1913, **14**, 539.

ROSENFELD, G. *Die Grundgesetze der Acetonurie und ihre Behandlung.* Centralbl. f. innere Med., 1895, **16**, 1233.

SABATIER, P. *Hydrogénation et deshydrogénations par catalyse* (Vortrag, gehalten vor der Deutschen Chem. Ges. am 13. Mai, 1911). Ber., 1911, **44**, 1984.

SALKOWSKI, E., AND H. SALKOWSKI. *Ueber das Verhalten der Phenylessigsäure und Phenylpropionsäure im Organismus.* Ber., 1879, **12**, 653.

SASAKI, T. *Ueber das Verhalten der Furfurpropionsäure im Tierkörper.* Biochem. Zeitschr., 1910, **25**, 272.

SCHOTTEN, C. *Ueber die flüchtigen Säuren des Pferdeharns und das Verhalten der flüchtigen Fettsäuren im Organismus.* Zeitschr. f. physiol. Chem.. 1882, **7**, 375.

SCHWARZ, L. *Ueber die Ausscheidung und Entstehung des Acetons.* Centralbl. f. Stoffwechsel u. Verdauungskrankheiten, 1900, **1**, 1.

SCHWENKEN, F. *Ueber das Verhalten der Acrylsäure im Organismus phlorhizindiabetischer Hunde.* Beitr. Physiol., 1914, **1**, 143.

THIERFELDER, H., AND C. P. SHERWIN. *Phenacetylglutamin, ein Stoffwechselprodukt des menschlichen Körpers nach Eingabe von Phenylessigsäure.* Ber., 1914, **47**, 2630.

THUNBERG, T. *Ueber die vitale Dehydrierung der Bernsteinsäure bei Abwesenheit von Sauerstoff.* Zentralbl. f. Physiol. 1916, **31**, **98**.

VON SCHRÖDER, W. *Ueber die Bildung des Harnstoffes.* Arch. f. exper. Path. u. Pharm., 1882, **15**, 364; 1885, **19**, 373.

WALDVOGEL, B. *Woraus und wo entsteht das Aceton?* Centralbl. f. innere Med., 1899, **20**, 729.

WALDVOGEL, R. *Zur Lehre von der Acetonurie.* Zeitschr. f. klin. Med., 1899, **38**, 506.

WALDVOGEL, R. *Die Acetonkörper.* F. Enke, Stuttgart, 1903.

WEGRZYNOWSKI, L. *Beiträge zur Lehre von der Entstehung der Oxalsäure im tierischen und menschlichen Organismus.* Zeitschr. physiol. Chem., 1913, **83**, 112.

WEINTRAUD, W. *Ueber die Beziehungen der Laevulinsäure zur Acetonurie.* Arch. f. exper. Path. u. Pharm. 1894, **34**, 367.

WISE, L. E. *Elimination of malates after subcutaneous injection of sodium malate.* Journ. Biol. Chem., 1916, **28**, 185.

WÖHLER, F. *Versuche über den Uebergang von Materien in den Harn.* Zeitschr. f. Physiol., 1824, **1**, 125.

ZELINSKI, N. *Ueber Dehydrogenisation durch Katalyse.* Ber., 1911, **44**, 3121.

REFERENCES TO CHAPTER III, PARTS I AND II.

The Oxidation and Reduction of the Amino Acids.

Abderhalden, E., and F. Samuely. *Der Abbau des Leucins und des Leucyl-leucins im Organismus des Hundes.* Zeitschr. f. physiol. Chem., 1906, **47**, 346.

Abderhalden, E., and A. Schittenhelm. *Studien über den Abbau razemischer Aminosäuren im Organismus des Hundes unter verschiedenen Bedingungen.* Zeitschr. f. physiol. Chem., 1907, **51**, 323.

Abderhalden, E., and M. Kempe. *Beitrag zur Kenntnis des Tryptophans und einiger seiner Derivate.* Zeitschr. f. physiol. Chem., 1907, **52**, 207.

Abderhalden, E., B. Bloch, and P. Rona. *Abbau einiger Dipeptide des Tyrosins und Phenylalanins bei einem Falle von Alkaptonurie.* Zeitschr. f. physiol. Chem., 1907, **52**, 435.

Abderhalden, E., and H. Einbeck. *Studien über den Abbau des Histidins im Organismus des Hundes.* Zeitschr. f. physiol. Chem., 1909, **62**, 322.

Abderhalden, E., and R. Massini. *Ueber das Verhalten von Mono-palmityl-l-tyrosin, Distearyl-l-tyrosin und von p-Amino-tyrosin im Organismus des Alcaptonurikers.* Zeitschr. f. physiol. Chem., 1910, **66**, 140.

Abderhalden, E., H. Einbeck, and J. Schmid. *Studien über den Abbau des Histidins im Organismus des Hundes.* Mitteilung II. Zeitschr. f. physiol. Chem., 1910, **68**, 395.

Abderhalden, E., and J. Markwalder. *Ueber die Verwertung einzelner Aminosäuren im Organismus des Hundes unter verschiedenen Bedingungen.* Zeitschr. f. physiol. Chem., 1911, **72**, 63.

Abderhalden, E., A. Furno, E. Goebel, and P. Strübel. *Weitere Studien über die Verwertung verschiedener Aminosäuren im Organismus des Hundes unter verschiedenen Bedingungen.* Zeitschr. f. physiol. Chem., 1911, **74**, 481.

Abderhalden, E. *Bildung von Homogentisinsäure nach Aufnahme großer Mengen von l-Tyrosin per os.* Zeitschr. f. physiol. Chem., 1912, **77**, 454.

Abderhalden, E., and P. Hirsch. *Die Bildung von Glykokoll im tierischen Organismus.* Zeitschr. f. physiol. Chem., 1912, **78**, 292.

Ackroyd, H., and F. G. Hopkins. *Feeding experiments with deficiencies in the amino acid supply. Arginine and histidine as possible precursors of purines.* Biochem. Journ., 1916, **10**, 551.

Araki, T. *Ueber die Bildung von Milchsäure und Glykose im Organismus bei Sauerstoffmangel.* Zeitschr. f. physiol. Chem., 1891, **15**, 325.

Araki, T. *Ueber die chemischen Aenderungen der Lebensprocesse in Folge von Sauerstoffmangel.* Zeitschr. f. physiol. Chem., 1894, **19**, 422.

Baer, J., and L. Blum. *Ueber den Abbau von Fettsäuren beim Diabetes mellitus.* Arch. f. exper. Path. u. Pharm., 1907, **56**, 92.

Baer, J., and L. Blum. *Ueber die Einwirkung chemischer Substanzen auf die Zuckerausscheidung und die Acidose I. und II.* Beitr. z. chem. Physiol. u. Path., 1907, **10**, 80; **11**, 101.

Bamberger, E. *Ueber das Verhalten paraalkylirter Phenole gegen das Caro'sche Reagens.* Ber., 1903, **36**, 2028.

BARGER, G., AND A. J. EWINS. *The synthesis of racemic Pr 2-methyltryptophane.* Biochem. Journ., 1917, **11**, 58.

BAUMANN, E., AND C. PREUSSE. *Zur Kenntnis der synthetischen Processe im Tierkörper.* Zeitschr. f. physiol. Chem., 1881, **5**, 309.

BAUMANN, E. *Zur Kenntnis der Phenylmercaptursäure, des Cystins und des Serins.* Ber., 1882, **15**, 1731.

BAUMANN, E. *Die aromatischen Verbindungen im Harn und die Darmfäulnis.* Zeitschr. f. physiol. Chem., 1866, **10**, 123.

BAUMANN, L., AND H. M. HINES. *The origin of creatine III.* Journ. Biol. Chem., 1918, **35**, 75.

BÉNECH, E., AND F. KUTSCHER. *Die Oxydationsprodukte des Arginins.* Zeitschr. f. physiol. Chem., 1901, **32**, 278.

BERGMANN, G. VON. *Die Ueberführung von Cystin in Taurin im tierischen Organismus.* Beitr. z. chem. Physiol. u. Path., 1903, **4**, 132.

BLENDERMANN, H. *Beiträge zur Kenntnis der Bildung und Zersetzung des Tyrosins im Organismus.* Zeitschr. f. physiol. Chem., 1882, **6**, 234.

BLUM, L. *Ueber das Schicksal des Cystins im Tierkörper.* Beitr. z. chem. Physiol. u. Path., 1904, **5**, 1.

BLUM, L. *Ueber den Einfluß des o-Tyrosins auf die Homogentisinsäureausscheidung beim Alkaptonuriker.* Beitr. z. chem. Physiol. u. Path., 1907, **11**, 142.

BLUM, L. *Ueber das Verhalten des p-Aminophenylalanin beim Alkaptonuriker.* Zeitschr. f. physiol. Chem., 1910, **67**, 192.

BORCHARDT, L., AND F. LANGE. *Ueber den Einfluß der Aminosäuren auf die Acetonkörperausscheidung.* Beitr. z. chem. Physiol. u. Path., 1907, **9**, 116.

BOSTOCK, G. R. *On Deamidization.* Biochem. Journ., 1911, **6**, 48.

BŌUGAULT, L., AND R. HEMMERLÉ. *Tautomerism of Phenylpyruvic acid.* Compt. r. de l'Acad. Sciences, 1915, **160**, 100.

BREINL, F., AND O. BAUDISCH. *Beiträge zur Kenntnis des oxydativen Abbaues der Keratine mit Wasserstoffsuperoxyd.* Zeitschr. f. physiol. Chem., 1907, **52**, 159.

CAMPS, R. *Ueber Liebig's Kynurensäure und das Kynurin und Synthese beider.* Zeitschr. f. physiol. Chem., 1901, **33**, 390.

CIAMICIAN, G. *Ueber die Entwickelung der Chemie des Pyrrols im letzten Vierteljahrhundert.* Ber., 1904, **37**, 4200 (cf. *Umwandlung der Pyrrols in Pyridinderivate*, p. 4231).

DAKIN, H. D. *The oxidation of amino-acids.* Journ. Biol. Chem., 1906, **1**, 171.

DAKIN, H. D. *The formation of glyoxylic acid.* Journ. Biol. Chem., 1906, **1**, 271.

DAKIN, H. D. *The oxidation of leucin, α-amido-isovaleric acid and of α-amido-n-valeric acid with hydrogen peroxide.* Journ. Biol. Chem., 1908, **4**, 63.

DAKIN, H. D. *Note on the oxidation of glutamic and aspartic acids by means of hydrogen peroxide.* Journ. Biol. Chem., 1909, **5**, 409.

DAKIN, H. D. *The mode of oxidation in the animal organism of phenyl derivatives of fatty acids.* Part. VI. *The fate of phenylalanine, phenyl-β-alanine, phenylglyceric acid and phenylacetaldehyde.* Journ. Biol. Chem., 1909, **6**, 235; 1910, **8**, 35.

DAKIN, H. D. *Experiments relating to the mode of decomposition of tyrosine and of related substances in the animal body.* Journ. Biol. Chem., 1910, **8**, 11.

DAKIN, H. D. *The fate of inactive tyrosine in the animal body together with some observations upon the detection of tyrosine and its derivatives in the urine. The synthesis and probable mode of formation of Blendermann's para-hydroxybenzyl-hydantoin.* Journ. Biol. Chem., 1910, **8**, 25.

DAKIN, H. D., AND A. J. WAKEMAN. *The catabolism of phenylalanine, tyrosine, and of their derivatives.* Journ. Biol. Chem., 1911, **9**, 139.

DAKIN, H. D. *The chemical nature of alcaptonuria.* Journ. Biol. Chem., 1911, **9**, 151.

DAKIN, H. D., AND A. J. WAKEMAN. *The catabolism of histidine.* Journ. Biol. Chem., 1911, **10**, 499.

DAKIN, H. D. *The fate of proline in the animal body.* Journ. Biol. Chem., 1913., **13**, 513.

DAKIN, H. D. *Studies on the intermediary metabolism of amino acids.* Journ. Biol. Chem., 1913, **14**, 321.

DAKIN, H. D., AND H. W. DUDLEY. *The mutual interconversion of α-amino acids, α-hydroxy acids and α-ketonic aldehydes.* Journ. Biol. Chem., 1913, **14**, 555; **15**, 127.

DAKIN, H. D., AND H. W. DUDLEY. *The fate of l-alanine in the glycosuric organism.* Journ. Biol. Chem., 1914, **17**, 451.

DAKIN, H. D., AND H. W. DUDLEY. *The formation of amino and hydroxy acids from glyoxals in the animal organism.* Journ. Biol. Chem., 1914, **18**, 29.

DAKIN, H. D. *Experiments on the interaction of hydroxy and amino acids with nitrophenylhydrazine with special reference to the formation of glyoxal derivatives.* Biochem. Journ., 1916, **10**, 313.

DAKIN, H. D. *On the oxidation of amino acids and of related substances with chloramine-T.* Biochem. Journ., 1917, **11**, 79.

DENIS, W. *The oxidation of the amino acids; I. Glycocoll and cystin.* Journ. Biol. Chem., 1911, **9**, 365.

DEWAR, J., AND A. GAMGEE. *Researches on the constitution and physiological relations of cystine.* Journ. of Anat. and Physiol., 1871 (2), **5**, 142.

DORNER, G. *Zur Bildung von Kreatin und Kreatinin im Organismus besonders der Kaninchen.* Zeitschr. f. physiol. Chem., 1907, **52**, 225.

ELLINGER, A. *Die Entstehung der Kynurensäure.* Zeitschr. f. physiol. Chem., 1904 **43**, 325.

ELLINGER, A. *Ueber die Constitution der Indolgruppe im Eiweiss.* III. Mitteilung. *Oxydation des Tryptophans zu β-Indolaldehyd.* Ber., 1906, **39**, 2515.

ELLINGER. A., AND Z. MATSUOKA. *Darstellung von α-Methyltryptophan und sein Verhalten im Tierkörper.* Zeitschr. f. physiol. Chem. 1914, **91**, 45.

ELLINGER, A., AND Z. MATSUOKA. *Zur Frage der Entstehung von Kynurensäure aus Tryptophan im Tierkörper.* Zeitschr. f. physiol. Chem., 1920, **109**, 259.

EMBDEN, H. *Beiträge zur Kenntnis der Alkaptonurie,* II. Mitteilung. Zeitschr. f. physiol. Chem., 1893, **18**, 304.

EMBDEN, G., AND H. SALOMON. *Ueber Alaninfütterungsversuche am pankreaslosen Hunde.* Beitr. z. chem. Physiol. u. Path., 1904, **5**, 507.

EMBDEN, G., AND H. SALOMAN. *Fütterungsversuche am pankreaslosen Hunde.* Beitr. z. chem. Physiol. u. Path., 1905, **6**, 63.

EMBDEN, G., H. SALOMON, AND F. SCHMIDT. *Ueber Acetonbildung in der Leber.* Beitr. z. chem. Physiol. u. Path., 1906, **8**, 129.

EMBDEN, G., AND A. MARX. *Ueber Acetonbildung in der Leber.* Beitr. z. chem. Physiol. u. Path.. 1908, **11**, 318.

EMBDEN, G., *Ueber das Verhalten der optisch isomeren Leucine in der Leber.* Beitr. z. chem. Physiol. u. Path., 1908, **11**, 348.

EMBDEN, G., AND A. MARX. *Ueber das Glykokoll im Harn.* Beitr. z. chem. Physiol. u. Path., 1908, **11**, 308.

EMBDEN, G., AND J. WIRTH. *Ueber die Hemmung der Acetessigsäurebildung in der Leber.* Biochem. Zeitschr., 1910, **27**, 1.

EMBDEN, G., AND E. SCHMITZ. *Ueber synthetische Bildung von Aminosäuren in der Leber.* Biochem. Zeitschr., 1910, **29**, 423; 1912, **38**, 393.

EMBDEN, G., AND F. KRAUS. *Ueber Milchsäurebildung in der künstlich durchströmten Leber.* Biochem. Zeitschr., 1912, **45**, 1.

EMBDEN, G., AND M. OPPENHEIMER. *Ueber den Abbau der Brenztraubensäure im Tierkörper.* Biochem. Zeitschr., 1912, **45**, 186; 1913, **55**, 335.

EMBDEN, G., AND K. BALDES. *Ueber Abbau des Phenylalanins im tierischen Organismus.* Biochem. Zeitschr., 1913, **55**, 301.

EMBDEN, G., AND S. ISAACS. *Ueber die Bildung von Milchsäure und Acetes in der diabetischen Leber.* Zeitschr. f. physiol. Chem., 1917, **99**, 297.

EPPINGER, H. *Ueber Melanurie.* Biochem. Zeitschr., 1910, **28**, 181.

ERLENMEYER, E., JR., AND J. KUNLIN. *Ueberführung der Glyoxylsäure in (durch Einwirkung von Ammoniak.* Ber., 1902, **35**, 2438.

FALTA, W., AND L. LANGSTEIN. *Die Entstehung von Homogentisinsäure aus alanin.* Zeitschr. f. physiol. Chem., 1903, **37**, 513.

FELDMAN, I., AND L. HILL. *The influence of oxygen inhalation on the lactic a duced during hard work.* Journ. Physiol., 1911, **42**, 439.

FISKER, C. H., AND J. B. SUMNER. *The importance of the liver in urea fo from amino acids.* Journ. Biol. Chem., 1914, **18**, 285.

FLATOW, L. *Ueber den Abbau von Aminosäuren im Organismus.* Zeitschr. f. Chem., 1910, **64**, 367.

FRIEDMANN, E. *Ueber die Konstitution des Cystins.* Beitr. z. chem. Physiol. 1 1903, **3**, 1.

FRIEDMANN, E. *Zur Kenntnis des Abbaues der Carbonsäuren im Tierkörper. Verhalten der normalen d-l-α-aminosäuren der Fettreihe im Orga des Hundes.* Beitr. z. chem. Physiol. u. Path., 1908, **11**, 151.

FRIEDMANN, E. *Zur Kenntnis des Abbaues der Carbonsäuren im Tierkörpe* teilung II. *Das Verhalten der normaten d-l-methylierten Aminosäu Organismus des Hundes.* Beitr. z. chem. Physiol. u. Path., 1908, **11**, 15

FRIEDMANN, E. *Zur Kenntnis des Abbaues der Carbonsäuren im Tierkörpe* teilung III. *Das Verhalten der verzweigten methylierten d-l-α-Aminc im Organismus des Hundes.* Beitr. z. chem. Physiol. u. Path., 1908, 1

FRIEDMANN, E. *Zur Kenntnis des Abbaues der Carbonsäuren im Tierkörper* teilung IV. *Das Verhalten der normalen dimethylierten d-l-α-Amino im Tierkörper.* Beitr. z. chem. Physiol. u. Path., 1908, **11**, 194.

FRIEDMANN, E. *Zur Kenntnis des Abbaues der Carbonsäuren im Tierkörper* teilung VI. *Zur Theorie der Homogentisinsäurebildung.* Beitr. z. chem. P u. Path., 1908, **11**, 304.

FRIEDMANN, E., AND C. MAASE. *Zur Kenntnis des Abbaues der Carbonsäur Tierkörper. IX. Verhalten des p-chlorphenyalanins, der p-chlorphenyl traubensäure und der p-chlorphenylmilchsäure im Tierkörper.* Biochem schr., 1910, **27**, 97.

FROMHERZ, K. *Ueber das Verhalten der p-Oxyphenyl-aminoessigsäure im körper.* Zeitschr. f. physiol. Chem., 1911, **70**, 351.

FROMHERZ, K., AND L. HERMANNS. *Ueber den Abbau aromatischer Aminosäur Tierkörper nach Versuchen am Normalen und am Alkaptonuriker.* Ze f. physiol. Chem., 1914, **91**, 194.

GARROD, A. E. *Inborn errors of metabolism.* Oxford Medical Publications,

GLAESNER, K., AND E. P. PICK. *Ueber Phlorhizindiabetes.* Beitr. z. chem. Phys Path., 1907, **10**, 473.

GRAEBE, C., AND O. SCHOTTEN. *Ueber das Verhalten der aromatischen Säuren Durchgang durch den tierischen Organismus.* Annalen, 1867, **142**, 3

GRUBE, K. *Zur Glykogenbildung in der Leber aus Formaldehyd.* Pflüger's A 1909, **126**, 585.

GRUNEWALD, I. *Observations on the Significance of glycollic acid, glyoxal, g aldehyde and aminoaldehyde in intermediary metabolism.* Journ. Biol. C 1918, **35**, 461.

HAAS, G. *Ueber das Schicksal der Glyoxylsäure im Tierkörper.* Biochem. Zeit 1912, **46**, 296.

HAAS, G. *Zur Frage der Glykokollbildung im Tierkörper.* Biochem. Zeitschr., **76**, 76.

HALSEY, J. T. *Concerning the Formation of Sugar from Leucine.* Amer. J Physiol., 1904, **10**, 229.

HAUSER, A. *Untersuchungen über die Kynurensäurebildung im Organismus.* Arch. f. exper. Path. u. Pharm., 1895, **36**, 1.

HÖCKENDORF, P. *Ueber den Einfluss einiger Alkohole, Oxy- und Aminosäuren der aliphatischen Reihe auf die Zucker- und Stickstoffausscheidung beim Phlorhizindiabetes des Hundes.* Biochem. Zeitschr., 1911, **23**, 281.

HOLLEMAN, A. F. *Action of hydrogen peroxide on 1, 2, diketones and on α-ketonic acids.* Proc. K. Akad. Wetensch, Amsterdam, 1904, **6**, 715.

HURTLEY, W. H., AND W. O. WOOTON. *The interaction of alloxan and glycine.* Trans. Chem. Soc., 1911, **99**, 288.

INOUYA, K. *Ueber die Entstehung des Kreatins im Tierkörper.* Zeitschr. f. physiol. Chem., 1912, **81**, 71.

JAFFÉ, M. *Untersuchungen über die Entstehung des Kreatins im Organismus.* Zeitschr. f. physiol. Chem., 1906, **48**, 430.

JAFFÉ, M. *Ueber die Aufspaltung des Benzolringes im Organismus. Das Auftreten von Muconsäure im Harn nach Darreichung von Benzol.* Zeitschr. f. physiol. Chem., 1909, **62**, 58.

JASTROWITZ, H. *Ueber Oxalsäurebildung im Organismus.* Biochem. Zeitschr., 1910, **28**, 34.

JANSEN, B. C. P. *The function of the liver in urea formation from amino acids.* Journ. Biol. Chem., 1915, **21**, 551.

DE JONG, A. W. K. *L'Action de l'Acide pyruvique sur son sel d'Ammonium.* Rec. trav. chim., 1900, **19**, 259.

KNOOP, F. *Ueber den physiologischen Abbau der Säuren und die Synthese einer Aminosäure im Tierkörper.* Zeitschr. f. physiol. Chem., 1910, **67**, 489.

KNOOP, F., AND E. KERTESS. *Das Verhalten von α-Aminosäuren und α-Ketonsäuren im Tierkörper.* Zeitschr. f. physiol. Chem., 1911, **71**, 153.

KNOOP, F. *Ueber Aminosäureabbau und Glykokollbildung.* Zeitschr. f. physiol. Chem., 1914, **89**, 151.

KNOPF, L. *Beiträge zur Kenntnis des Phlorhizindiabetes.* Arch. f. exper. Path. u. Pharm., 1903, **49**, 123.

KONDO, K. *Ueber synthetische Aminosäurebildung in der Leber.* Biochem. Zeitschr., 1912, **38**, 407.

KOSSEL, A., AND H. D. DAKIN. *Ueber die Arginase.* Zeitschr. f. physiol. Chem., 1904, **41**, 321.

KOSSEL, A., AND H. D. DAKIN. *Weitere Untersuchungen über fermentative Harnstoffbildung.* Zeitschr. f. physiol. Chem., 1904, **42**, 181.

KOTAKE, Y. *The formation of oxyphenylacetic acid in the organism and its relation to tyrosine catabolism.* Journ. Biol. Chem., 1919, **35**, 53.

KOWALEVSKI, K. *Ueber das Schicksal des Histidins im Körper des Hundes.* Biochem. Zeitschr., 1910, **23**, 1.

KUMAGAI, T., AND R. WOLFFENSTEIN. *Ueber die Einwirkung von Kaliumpersulfat auf p-Kresol.* Ber., 1908, **41**, 297.

KUTSCHER, F. *Die Oxydationsprodukte des Arginins.* II. Mitteilung. Zeitschr. f. physiol. Chem., 1901, **32**, 413.

KWISDA, A. *Einwirkung von Jodwasserstoffsäure auf einige Aminosäuren.* Monatshefte f. Chem., 1891, **12**, 419.

LANG, S. *Ueber Desamidierung im Tierkörper.* Beiträge z. chem. Physiol. u. Path. 1904, **5**, 321.

LANGHELD, K. *Ueber den Abbau der α-Aminosäuren zu fetten Aldehyden mittelst Natriumhypochlorit.* Ber., 1909, **42**, 392.

LIEBIG, J. *Ueber die Oxydation organischer Verbindungen.* Annalen, 1849, **70**, 311.

LIPPICH, F. *Ueber Uraminosäuren.* Ber., 1908, **41**, 2953, 2974.

LUSK, G. *The production of sugar from glutamic acid ingested in phlorhizin glycosuria.* Amer. Journ. Physiol., 1908, **22**, 174 (see also Ringer and Lusk).

LUSK, G. *The influence of the ingestion of amino acids upon metabolism.* Journ. Biol. Chem., 1912, **13**, 155.

MAGANINI, G. *Ueber die Verwandlung der Indole in Chinolinderivate.* Ber., 1887, **20**, 2608.

MAYER, P. *Experimentelle Beiträge zur Frage des intermediären Stoffwechsels der Kohlehydrate.* I. Mitt. *Ueber Aethylenglykol und Glykolaldehyd.* Zeitschr. f. physiol. Chem., 1903, **38**, 135.

MAYER, P. *Ueber das Verhalten der Diaminopropinsäure im Tierkörper.* Zeitschr. f. physiol. Chem., 1904, **42**, 59.

MANDEL, A. R., AND G. LUSK. *Lactic acid in intermediary metabolism.* Amer. Journ. Physiol., 1906, **16**, 129.

MEYER, F. *Ueber Alkaptonurie.* Deutsches Archiv. f. klin. Med., 1901, **70**, 443.

MÖRNER, K. A. H. *Brenztraubensäure unter den Spaltungsprodukten der Proteinstoffe.* Zeitschr. f. physiol. Chem., 1904, **42**, 121.

NEUBAUER, O., AND W. FALTA. *Ueber das Schicksal einiger aromatischer Säuren bei der Alkaptonurie.* Zeitschr. f. physiol. Chem., 1904, **42**, 81.

NEUBAUER, E. *Ueber das Schicksal der Milchsäure bei normalen und phosphorvergifteten Tieren.* Arch. f. exper. Path., 1909, **61**, 387.

NEUBAUER, O. *Ueber den Abbau der Aminosäuren im gesunden und kranken Organismus.* Deutsch. Arch. f. klin. Med., 1909, **95**, 211.

NEUBAUER, O., AND W. GROSS. *Zur Kenntnis des Tyrosinabbaues in der künstlich durchbluteten Leber.* Zeitschr. f. physiol. Chem., 1910, **67**, 219.

NEUBAUER O., AND H. FISCHER. *Beiträge zur Kenntnis der Leberfunktionen (Desaminierung, Reduktion und Kohlensäureabspaltung in der künstlich durchbluteten Leber).* Zeitschr. f. physiol. Chem., 1910, **67**, 230.

NEUBAUER, O., AND O. WARBURG. *Ueber eine Synthese mit Essigsäure in der künstlich durchbluteten Leber.* Zeitschr. f. physiol. Chem., 1910, **70**, 1.

NEUBAUER, O., AND O. FROMHERZ. *Ueber den Abbau der Aminosäuren bei der Hefegärung.* Zeitschr. f. physiol. Chem., 1911, **70**, 326.

NEUBERG, C., AND L. LANGSTEIN. *Ein Fall von Desamidierung im Tierkörper, zugleich ein Beitrag zur Frage nach der Herkunft des Glykogens.* Vorhandl. d. physiol. Gesellsch. Berlin, His-Engelmanns Arch., physiol. Abt., 1903, Supplementband, 514.

NEUBERG, C. *Chemische Umwandlungen durch Strahlenarten.* I. Mitteilung. *Katalytische Reaktionen des Sonnenlichtes.* Biochem. Zeitschr., 1908, **13**, 305.

NEUBERG, C. *Abbau einiger Di- und Oxyaminosäuren.* Biochem. Zeitschr., 1909, **20**, 531.

OPPENHEIMER, S. *Ueber die Ausscheidung von Alanin durch den Harn.* Beitr. z. chem. Physiol. u. Path., 1907, **10**, 273.

PARNAS, J. *Ueber das Schicksal der stereoisomeren Milchsäure im Organismus des normalen Kaninchens.* Biochem. Zeitschr., 1911, **38**, 53.

RIESSER, O. *Theoretisches und Experimentelles zur Frage der Kreatinbildung im tierischen Organismus.* Zeitschr. f. physiol. Chem., 1913, **86**, 415.

RIESSER, O. *Weitere Beiträge zur Frage der Kreatinbildung aus Cholin und Betain.* Zeitschr. f. physiol. Chem., 1914, **90**, 221.

RINGER, A. I., AND G. LUSK. *Ueber die Entstehung von Dextrose aus Aminosäuren bei Phlorhizinglykosurie.* Zeitschr. f. physiol. Chem., 1910, **66**, 106.

ROSENMUND, K. W., AND H. DORNSAFT. *Ueber Oxy- und Dioxyphenylserine und die Muttersubstanz des Adrenalins.* Ber. 1919, **52**, 1734.

RYFFEL, J. H. *Experiments on lactic acid formation in man.* Journ. Physiol., 1910, **39**, Proc. XXIX.

SACHS, F. *Ueber den Chemismus des Leucinabbaues in der Leber.* Biochem. Zeitschr., 1910, **27**, 27.

SALASKIN, S. *Ueber die Bildung von Harnstoff in der Leber der Säugetiere aus Aminosäuren der Fettreihe.* Zeitschr. f. physiol. Chem., 1898, **25**, 128.

SALKOWSKI, E. *Ueber die Bildung des Harnstoffes im Tierkörper.* Ber., 1875, **7**, 116.

SALKOWSKI, E. *Ueber die Entstehung der Schwefelsäure und da Verhalten des Taurins im tierischen Organismus.* Virchow's Archiv, 1873, **58**, 460.

SALKOWSKI, E., AND H. SALKOWSKI. *Ueber das Verhalten der Phenylessigsäure und Phenylpropionsäure im Organismus.* Ber., 1879, **12**, 653.

SANSUM, W. D., AND R. T. WOODYATT. *Glycollic aldehyde in phlorhizinised dogs.* Journ. Biol. Chem., 1914, **17**, 521.

SASSA, R. *Zur Frage der Glykokollsynthese im Organismus.* Biochem. Zeitschr., 1914, **59**, 353.

SCHMIDT, A. *Ueber das Verhalten einiger Chinolinderivate im Tierkörper mit Rücksicht auf die Bildung von Kynurensäure.* Inaug.-Dissertation, Königsberg, 1884.

SCHMIDT, C. L. A., AND E. G. ALLEN. *The elimination of taurine administered to man.* Journ. Biol. Chem., 1920, **42**, 55.

SCHMITZ, E. *Ueber das Verhalten der p-Oxyphenyl-α-milchsäure und der p-Oxyphenylbrenztraubensäure in der überlebenden Leber.* Biochem. Zeitschr., 1910, **28**, 117.

SCHOTTEN, C. *Ueber die Quelle der Hippursäure im Harn.* Zeitschr. f. physiol. Chem., 1883, **8**, 60.

SCHOTTEN, C. *Ueber das Verhalten des Tyrosins und der aromatischen Oxysäuren im Organismus.* Zeitschr. f. physiol. Chem. 1882, **7**, 23.

SCHULZEN, O., AND M. NENCKI. *Die Vorstufen des Harnstoffes im tierischen Organismus.* Zeitschr. f. Biol., 1872, **8**, 124.

VAN SLYKE, D. D., AND G. M. MEYER. *The fate of protein digestion products in the body.* Journ. Biol. Chem., 1913, **16**, 197, 213, 231.

SOLOMIN, P. *Zur Kenntnis der Kynurensäure.* Zeitschr. f. physiol. Chem., 1897, **23**, 407.

STOLTÉ, K. *Ueber das Schicksal der Monaminosäuren im Tierkörper nach Einführung in die Blutbahn.* Beitr. z. chem. Physiol. u. Path., 1903, **5**, 15.

STRECKER, A. *Notiz über eine eigentümliche Oxydation durch Alloxan.* Annalen, 1862, **123**, 363.

SUWA, A. *Ueber das Schicksal der N-freien Abkömmlinge der aromatischen Aminosäuren im normalen Organismus.* Zeitschr. f. physiol. Chem., 1911, **72**, 113.

THOMAS, K., AND M. H. G. GOERNE. *Ueber die Herkunft des Kreatins im tierischen Organismus.* Zeitschr. f. physiol. Chem., 1914, 92, 163.

THOMPSON, W. H. *The physiological effects of peptone and allied products.* Parts VI and VII. *The metabolism of arginine.* Journ. Physiol., 1905, **32**, 137; **33**, 106.

THOMPSON, W. H. *The metabolism of arginine.* Journ. Physiol., 1917, **51**, 111.

WIRTH, J. *Ueber den Abbau des Isoleucins in der Leber.* Biochem. Zeitschr., 1910, **27**, 20.

WOHLGEMUTH, J. *Ueber die Herkunft der schwefelhaltigen Stoffwechselprodukte im tierischen Organismus.* Zeitschr. f. physiol. Chem., 1903, **40**, 81.

WOHLGEMUTH, J. *Ueber das Verhalten stereoisomerer Substanzen im tierischen Organismus.* II. *Die inactiven Monoaminosäuren.* Ber., 1905, **38**, 2064.

WOLKOW, M., AND E. BAUMANN. *Ueber das Wesen der Alkaptonurie.* Zeitschr. f. physiol. Chem., 1891, **15**, 228.

REFERENCES TO CHAPTER III, PART III.

OXIDATION AND REDUCTION OF AMINO ACIDS BY MICRO-ORGANISMS.

ABDERHALDEN, E., AND Y. TERUUCHI. *Kulturversuche mit Aspergillus niger auf einige Aminosäuren und Peptiden.* Zeitschr. f. physiol. Chem., 1906, **47**, 394.

ACKERMANN, D. *Ein Fäulnisversuch mit Arginin.* Zeitschr. f. physiol. Chem., 1908, **56**, 305.

ACKERMANN, D. *Ueber die Entstehung von Fäulnisbasen.* Zeitschr. f. physiol. Chem 1909, **60**, 482.

ACKERMANN, D. *Ueber den bakteriellen Abbau des Histidins.* Zeitschr. f. physiol Chem., 1910, **65**, 504.

ACKERMANN, D. *Ueber ein neues auf bakteriellem Wege gewinnbares Aporrhegma.* Zeitschr. f. physiol. Chem., 1910, **69**, 273.

ACKERMANN, D. *Ueber das β-Alanin als bakterielles Aporrhegma.* Zeitschr. f. Biol., 1911, **56**, 87.

ACKERMANN, D. *Die Sprengung des Pyrrolidinringes durch Bakterien.* Zeitschr. f. Biol., 1911, **57**, 104.

ACKERMANN, D. *Ueber den fermentativen Abbau des Kreatinins.* Zeitschr. f. Biol., 1913, **62**, 209; **63**, 78.

ACKERMANN, D., AND F. KUTSCHER. *Ueber die Aporrhegmen.* Zeitschr. f. physiol. Chem., 1910, **69**, 265.

AMATSU, H., AND M. SSUDJI. *Ueber den Abbau von d-l-Phenylalanin durch B. proteus und B. subtilis.* Act. Schol. Med. Univ. Kioto., 1918, **2**, 447.

BARGER, G. *Bemerkungen zum Aufsatz des Herrn D. Ackermann „Ueber die Entstehung von Fäulnisbasen".* Zeitschr. f. physiol. Chem., 1909, **61**, 188.

BARGER, G. *Isolation and synthesis of p-hydroxyphenylethylamine, an active principle of ergot soluble in water.* Trans. Chem. Soc., 1909, **95**, 1123.

BARGER, G., AND G. S. WALPOLE. *Isolation of the pressor principles from putrid meat.* Journ. Physiol., 1909, **38**, 343.

BAUMANN, E. *Ueber die Bildung von Hydroparacumarsäure aus Tyrosin.* Ber., 1879, **12**, 1450.

BAUMANN, E. *Zur Kenntnis der aromatischen Substanzen des Tierkörpers.* Zeitschr. f. physiol. Chem., 1883, **7**, 282.

BAUMANN, E. *Zur Kenntnis der aromatischen Substanzen des Tierkörpers.* Zeitschr. f. physiol. Chem., 1883, **7**, 553.

BORCHARDT, L. *Fäulnisversuche mit Glutamin- und Asparaginsäure.* Zeitschr. f. physiol. Chem., 1909, **59**, 96.

BRASCH, W., AND C. NEUBERG. *Biochemische Umwandlung der Glutaminsäure in n-Buttersäure.* Biochem. Zeitschr., 1908, **13**, 299.

BRASCH, W. *Ueber den bakteriellen Abbau primärer Eiweissspaltprodukte.* Biochem. Zeitschr., 1909, **18**, 380.

BRASCH, W. *Weitere Untersuchungen über den bakteriellen Abbau primärer Eiweissspaltungsprodukte.* Biochem. Zeitschr. 1909, **22**, 403.

BRIEGER, L. *Die Ptomaine.* III. Hirschwald, Berlin.

EHRLICH, F. *Ueber die Entstehung des Fuselöls.* Zeitschr. d. Verb. d. Deutsch. Zuckerind., 1905, **55**, 539.

EHRLICH, F. *Ueber eine Methode zur Spaltung racemischer Aminosäuren mittelst Hefe.* Biochem. Zeitschr., 1906, **1**, 8.

EHRLICH, F. *Die chemischen Vorgänge bei der Hefegährung.* Biochem. Zeitschr., 1906, **2**, 52.

EHRLICH, F. *Zur Frage der Fuselölbildung der Hefe.* Ber., 1906, **39**, 4072.

EHRLICH, F. *Ueber die Bedingungen der Fuselölbildung und über ihren Zusammenhang mit dem Eiweissaufbau der Hefe.* Ber., 1907, **40**, 1027.

EHRLICH, F. *Ueber die Spaltung racemischer Aminosäuren mittelst Hefe.* II. Biochem. Zeitschr., 1908, **8**, 438.

EHRLICH, F. *Ueber die Entstehung der Bernsteinsäure bei der alkoholischen Gährung.* Biochem. Zeitschr., 1909, **18**, 391.

EHRLICH, F., AND P. PISTSCHIMUKA. *Ueberführung von Aminen in Alkohole durch Hefe und Schimmelpilze.* Ber., 1912, **45**, 1006.

ELLINGER, A. *Ueber die Entstehung von Fäulnisbasen.* Zeitschr. f. physiol. Chem., 1910, **65**, 394.

EMMERLING, O. *Oxalsäurebildung durch Schimmelpilze.* Centralbl. f. Bakt., 1903 (2), **10**, 273.

HIRAI, K. *Ueber Tyrosolbildung aus l-Tyrosin durch Bakterien.* Act. Schol. Med.. Univ. Kioto, 1917, **2**, 425.

HIRAI, K. *Ueber die Bildung der α-β-Iminazolylmilchsäure aus l-Histidin durch Bakterien.* Act. Schol. Med. Univ. Kioto, 1919, **3**, 1.

HOPKINS, F. G., AND S. W. COLE. *A Contribution to the Chemistry of Proteids.* Part II. *The Constitution of Tryptophane and the Action of Bacteria upon it.* Journ. Physiol., 1903, **29**, 451.

LINTNER, C. J., AND H. J. v. LIEBIG. *Ueber die Reduktion des Furfurols durch Hefe bei der alkoholischen Gährung.* Zeitschr. f. physiol. Chem., 1911, **72**, 449.

NENCKI, M. *Ueber die Bildung des Indols aus dem Eiweiß.* Ber., 1875, **8**, 336.

NENCKI, M. *Ueber das Indol.* Ber. 1875, **8**, 722.

NENCKI, M. *Ueber den chemischen Mechanismus der Fäulnis.* Journ. f. prakt. Chem., 1878, **17**, 105.

NENCKI, M. *Ueber die Zersetzung der Gelatine und des Eiweisses bei der Fäulnis mit Pankreas.* Gesammelte Arbeiten. Vol. I., p. 181 (F. Vieweg u. Sohn, 1904) (Festgabe an G. Valentin).

NEUBAUER, O., AND K. FROMHERZ. *Ueber den Abbau der Aminosäuren bei der Hefegärung.* Zeitschr. f. physiol. Chem., 1911, **70**, 326.

NEUBERG, C., AND E. ROSENBERG. *Ueber die bei der Eiweissfäulnis auftretenden Fettsäuren sowie über die optisch aktive Valeriansäure und Kapronsäure.* Biochem. Zeitschr., 1907, **7**, 178.

NEUBERG, C., AND L. KARCZAG. *Verhalten von d-l-α-Aminoisovaleriansäure bei der Fäulnis.* Biochem. Zeitschr., 1909, **18**, 435.

NEUBERG, C., AND C. KAPPEZZUOLI. *Biochemische Umwandlung von Asparagin und Asparaginsäure in Propionsäure und Bernsteinsäure.* Biochem. Zeitschr., 1909, **18**, 424.

NEUBERG, C. *Verhalten von racemischer Glutaminsäure bei der Fäulnis.* Biochem. Zeitschr., 1909, **18**, 431.

NEUBERG, C., AND A. HILDESHEIMER. *Ueber zuckerfreie Hefegärungen.* I. Biochem. Zeitschr., 1911, **31**, 171.

NEUBERG, C., AND L. TIR. *Ueber zuckerfreie Hefegärungen.* II. Biochem. Zeitschr., 1911, **32**, 323.

NEUBERG, C., AND L. KARCZAG. *Ueber zuckerfreie Hefegärungen.* Biochem. Zeitschr., 1911, **36**, 60.

NEUBERG, C., AND L. KARCZAG. *Ueber zuckerfreie Hefegärungen.* IV. *Carboxylase, ein neues Enzym der Hefe.* Biochem. Zeitschr., 1911, **36**, 68.

NEUBERG, C., AND L. KARCZAG. *Ueber zuckerfreie Hefegärungen.* V. *Zur Kenntnis der Carboxylase.* Biochem. Zeitschr., 1911, **36**, 76.

NEUBERG, C. *Biochemische Umwandlung von α-Pyrrolidincarbonsäure in n-Valeriansäure und δ-Aminovaleriansäure.* Biochem. Zeitschr., 1911, **37**, 490.

NEUBERG, C. *Ueber die Herkunft der optisch-aktiven Valeriansäure bei der Eiweissfäulnis.* Biochem. Zeitschr., 1911, **37**, 501.

RAISTRICK, H. *The conversion of histidine into urocanic acid by bacteria of the coli-typhosus group.* Biochem. Journ., 1917, **11**, 71.

SALKOWSKI, E. AND H. *Weitere Beiträge zur Kenntnis der Fäulnisprodukte des Eiweiss.* Ber., 1879, **12**, 648; 1880, **13**, 189.

SALKOWSKI, E. AND H. *Ueber die Entstehung der Homologen der Benzoesäure bei der Fäulnis.* Zeitschr. f. physiol. Chem., 1883, **7**, 450.

SALKOWSKI, E. AND H. *Zur Kenntnis der Eiweissfäulnis.* II. *Die Skatolcarbonsäure.* Zeitschr., f. physiol. Chem., 1884, **9**, 8.

SALKOWSKI, E. *Zur Kenntnis der Eiweissfäulnis.* III. *Ueber die Bildung der nicht hyrdroxylirten aromatischen Säuren.* Zeitschr. f. physiol. Chem., 1885, **9**, 491.

SALKOWSKI, E. *Ueber die Bildung von Skatolessigsäure bei der Eiweißfäulnis.* Zeitschr. f. physiol. Chem., 1899, **27**, 302.

SALKOWSKI, H. *Ueber δ-Amidovaleriansäure.* Ber., 1898, **31**, 776.

SALKOWKSI, E. *Zur Kenntnis der Eiweissfäulnis.* I. *Ueber die Bildung des Indols und Skatols.* Zeitschr. f. physiol. Chem., 1884, **8**, 417.

SASAKI, T. *Ein optisch differenter Abbau des l-Tyrosins durch Proteus bzw. Subtilis.* Act. Schol. Med. Univ. Kioto., 1915, **1**, 103.

TRAETTA-MOSCA, F. *La Fermentazione della Tyrosina.* Gaz. chim. Ital., 1910, **40**, 86.

TSUDJI, M. *Ueber den pakteriellen Abbau von Tyrosin mit besonderer Berücksichtigung des stereochemischen Verhaltens der Abbauprodukte.* Act. Schol. Med. Univ. Kioto., 1916, **2**, 115; 1917, **1**, 439.

WEYL, T. *Spaltung von Tyrosin durch Fäulnis.* Zeitschr. f. physiol. Chem., 1879, **3**, 312.

WOHLGEMUTH, J. *Ueber die Herkunft der schwefelhaltigen Stoffwechselprodukte im tierischen Organismus.* II. Zeitschr. f. physiol. Chem., 1905, **43**, 469.

RERERENCES TO CHAPTER IV.

THE CARBOHYDRATES.

ANDERSON, R. J., AND A. W. BOSWORTH. *The utilisation of inosite in the animal organism.* Journ. Biol. Chem., 1916, **25**, 399.

BALEAR, J. O. *The physiological action of glucal.* Journ. Biol. Chem., 1916, **26** 163.

BALDES, K., AND F. SIDERSTEIN. *Ueber synthetische Zuckerbildung in der künstlich durchströmten Leber.* Zeitschr. f. physiol. Chem., 1917, **100**, 34.

BERGELL, P. *Verhalten der l-Arabinose im normalen und diabetischen Organismus.* Internat. Beiträge z. innern Med., 1902, gew. E. V. Leyden, II, 401.

BEYSEL, W., AND W. LÖB. *Die katalytische Beeinflussung der oxydativen Glykolyse.* Biochem. Zeitschr., 1915, **68**, 368.

BIBERFELD, J. *Zum Verhältnis der Glucuronsäure im Organismus.* Biochem. Zeitschr., 1914, **65**, 479.

CREMER, M. *Entsteht aus Glyzerin und Fett im Körper des höheren Tieres Traubenzucker?* Sitzungsber. d. Ges. Morph. u. Physiol. in München, 1902, Heft 2. Maly's Jahresbericht, 1903, **33**, 604.

CREMER, M. *Ueber die Verwertung der Rhamnose im tierischen Organismus und einige damit zusammenhängende Fragen der Physiologie der Kohlehydrate.* Zeitschr. f. Biologie, 1902, **32**, 428.

CREMER, M. *Physiologie des Glykogens.* Ergebnisse der Physiologie, 1902, **1**, 803.

DAKIN, H. D., AND H. W. DUDLEY. *The interconversion of α-amino acids, α-hydroxy acids and α-ketonic acids.* Part II. Journ. Biol. Chem., 1913, **15**, 127.

DAKIN, H. D., AND H. W. DUDLEY. *An enzyme concerned with the formation of hydroxy acids from ketonic aldehydes.* Journ. Biol. Chem., 1913, **14**, 321, 423; **15**, 463; **16**, 505.

EMBDEN, G., AND FR. KRAUS. *Beitrag zur Lehre vom Abbau der Kohlehydrate im Tierkörper.* Verh. d. Kongr. f. inn. Med., 1909, **26**, 350.

EMBDEN, G., K. BALDES AND E. SCHMITZ. *Ueber den Chemismus der Milchsäurebildung aus Traubenzucker.* Biochem. Zeitschr., 1912, **45**, 108.

EMBDEN, G., E. SCHMITZ AND K. BALDES. *Ueber den Chemismus der Glycerinbildung im Tierkörper.* Biochem. Zeitschr., 1912, **45**, 174.

EMBDEN, G., AND W. GRIESBACH. *Ueber Milchsäurebildung in der isolierten Leber. I. Ueber den Abbau der α-Sorbose. II. Ueber das Schicksal des α-Sorbits und einiger anderer Hexite.* Zeitschr. f. physiol. Chem., 1914, **91**, 251.

EMBDEN, G., E. SCHMITZ, AND M. WITTENBERG. *Ueber synthetische Zuckerbildung in der künstlich durchströmten Leber.* Zeitschr. f. physiolog. Chem. 1913, **88**, 210.

EMBDEN, G., W. GRIESBACH, AND F. LAQUER. *Ueber den Abbau von Hexosephosphorsäure und Lactacidogen durch einige Organpresssäfte.* Zeitschr. f. physiol. Chem., 1914, **93**, 124.

FABIAN, E. *Ueber das Verhalten des salzsauren Glycosamins im Tierkörper.* Zeitschr. f. physiol. Chem., 1899, **27**, 167.

GLATTFELD, J. W. E. *On the oxidation of α-glucose in alkaline solution by air as well as by hydrogen peroxide.* Amer. Chem. Journ., 1913, 50, 135.

GRIESBACH, W. *Ueber Milchsäurebildung aus Kohlenhydrat im lackfarbenen Blut.* Biochem. Zeitschr., 1913, **50**, 457.

HARDEN, A., AND H. MACLEAN. *On the alleged presence of an alcoholic enzyme in animal tissues and organs.* Journ. Physiol., 1911, **42**, 64.

HEWITT, J. A., AND J. PRYDE. *The metabolism of carbohydrates.* Part I. Biochem. Journ., 1920, **14**, 395.

HILDEBRANDT, H. *Ueber einige Synthesen im Tierkörper.* Arch. f. exper. Path. u. Pharm., 1900, **44**, 278.

HILDEBRANDT, H. *Ueber eine experimentelle Stoffwechselabnormität.* Zeitschr. f. physiol. Chem., 1902, **35**, 141.

HORNEMANN, H. *Ueber die Bildung von Wein- und Traubensäure bei der Einwirkung von Salpetersäure auf Kohlenhydrate.* Journ. f. prakt. Chem., 1863, **89**, 283.

ISAAC, S. *Ueber die Umwandlung von Lävulose in Dextrose in der künstlich durchströmten Leber.* Zeitschr. f. physiol. Chem. 1914, **89**, 78.

JAKSCH, R. VON. *Ueber die alimentäre Pentosurie der Diabetiker.* Deutsch. Arch. f. klin. Med., 1899, **63**, 612.

JAKSCH, R. VON. *Ueber alimentäre Pentosurie.* Zeitschr. f. Heilkunde, 1899, **20**, 195.

JENSEN, P. B. *Die Zersetzung des Zuckers während des Respirationsprozesses* (vorläufige Mitteilung). Ber. deutsch. bot. Ges., 1908, **26a**, 666.

JENSEN, P. B. *Sukkersönderdelingen under Respirationsprocessen hos höjere Planter.* Dissertation Köbenhavn (H. H. Hagerup), 1910.

JOLLES, A. *Ueber eine neue Bildungsweise der Glucuronsäure.* Biochem. Zeitschr., 1911, **34**, 242.

KILIANI, H. AND S. KLEEMANN. *Umwandlung der Gluconsäure in normales Caprolacton bzw. normale Capronsäure.* Ber., 1884, **17**, 1296.

KNOPP, F., AND A. WINDAUS. *Ueber Beziehungen zwischen Kohlehydraten und stickstoffhaltigen Produkten des Stoffwechsels.* Beitr. z. chem. Physiol u. Path., 1905, **6**, 392.

KRASKE, B. *Ueber Milchsäurebildung im Blute.* Biochem. Zeitschr., 1912, **45**, 81.

LENEL, R. *Die Ausnutzung des α-Glucoheptonsäurelacton beim Diabetiker und Nichtdiabetiker.* Arch. exp. Path. u. Pharm., 1914, **77**, 335.

LEVENE, P. A., AND G. M. MEYER. *On the combined action of muscle plamas and pancreas extract on glucose and maltose.* Journ. Biol. Chem., 1911, **9**, 97.

LEVENE, P. A., AED G. M. MEYER. *The action of leucocytes on glucose.* Journ. Biol. Chem. 1912, **11**, 361.

LEVENE, P. A., AND G. M. MEYER. *On the action of leucocytes on some hexoses and pentoses.* Journ. Biol. Chem., 1913, **14**, 149.

LEVENE, P. A., AND G. MEYER. *On the action of tissues on hexoses.* Biol. Chem., 1913, **15**, 65.

LINDEMANN, L., AND R. MAY. *Die Verwertung von Rhamnose vom normalen und vom diabetischen menschlichen Organismus.* Deutsches Archiv f. klin. Med., 1896, **56**, 282.

LÖB, W. *Zur Kenntnis der Zuckerspaltungen.* I. Mitteilung. *Die Einwirkung von Zinkkarbonat auf Formaldehydlösungen.* Biochem. Zeitschr., 1908, **12**, 78.

LÖB, W. *Zur Kenntnis der Zuckerspaltungen.* III. Mitteilung. *Die Elektrolyse des Traubenzuckers.* Biochem. Zeitschr., 1909, **17**, 132.

LÖB, W. *Zur Kenntnis der Zuckerspaltungen.* V. Mitteilung. *Die Umkehrung der Zuckersynthese.* Biochem. Zeitschr., 1909, **20**, 516.

LÖB, W. *Zur Kenntnis der Zuckerspaltungen.* VI. Mitteilung. *Die elektrolytische Reduktion des Traubenzuckers.* Biochem. Zeitschr., 1909, **22**, 103.

LÖB, W. *Beiträge zur Frage der Glykolyse.* I. Biochem. Zeitschr., 1910, **29**, 316.

LÖB, W., AND G. PULVERMACHER. *Zur Kenntnis der Zuckerspaltungen.* VII. Mitteilung. ... *ehrung der Zuckersynthese.* Biochem. Zeitschr., 1909, **23**, 10.

... *ie Milchsäurebildung aus Traubenzucker, Glyzerinaldehyd und im Rinder- und Schweineblut.* Biochem. Zeitschr., 1913, **50**, 451.

LOEWI, O. *Ueber den Einfluss des Kampfers auf die Grösse der Zuckerausscheidung im Phlorhizindiabetes.* Arch. f. exper. Path. u. Pharm., 1902, **47**, 56.

LUCHSINGER, B. *Beiträge zur Physiologie und Pathologie des Glykogens.* Dissertation, Zürich, 1875.

LÜTHJE, H. *Die Zuckerbildung aus Glyzerin.* Deutsch. Arch. f. klin. Med., 1904, **80**, 98.

MANDEL, J. A., AND H. C. JACKSON. *On the origin of glycuronic acid.* Amer. Journ. Physiol., 1902, **8**, 13 (Proc.).

MANDEL, J. A., AND G. LUSK. *Lactic acid in intermediary metabolism.* Amer. Journ. Physiol., 1906, **16**, 129.

MAYET, P. *Experimentelle Untersuchungen über den Abbau des Zuckers im Tierkörper.* Verhdlg. d. Kongr. f. inner. Med., 1901, **19**, 393.

MAYER, P. *Ueber unvollkommene Zuckeroxydation im Organismus.* Deutsche med. Woch., 1901, **16**, 243; **17**, 262.

MAYER, P. *Ueber das Verhalten der d-Glukonsäure im Organismus.* Ber., 1901, **34**, 492.

MAYER, P. *Experimentelle Untersuchungen über Kohlenhydratsäuren.* Zeitschr. f. klin. Med., 1902, **47**, 68.

MAYER, P. *Ueber einige Fragen des intermediären Kohlehydratstoffwechsels.* (Vortrag.) Wien. Med. Woch., 1904, **54**, 881, 917.

MAYER, P. *Neuere Untersuchungen über die Glukuronsäure.* Sammelreferat. Biochem. Zentralbl. 1903, **1**, 377.

MAYER, P. *Experimentelle Beiträge zur Frage des intermediären Stoffwechsels der Kohlehydrate. Ueber Aethylenglykol und Glykolaldehyd.* Zeitschr. f. physiol. Chem., 1903, **38**, 135.

MERING, VON. *Zur Glykogenbildung in der Leber.* Pflüger's Archiv, 1877, **14**, 274.

NEF, J. U. *Ueber das Verhalten der Zuckerarten gegen die Fehlingsche Lösung sowie gegen andere Oxydationsmittel.* Annalen, 1907, **357**, 214.

NEF, J. U. *Dissociationsvorgänge in der Zuckergruppe.* Liebig's Ann., 1913, **403**, 204.

NEUBAUER, O., AND K. FROMHERZ. *Ueber den Abbau der Aminosäuren bei der Hefegärung.* Zeitschr. f. physiol. Chem., 1910, **70**, 326.

NEUBERG, C. *Ueber die Pentosen des Tierkörpers.* Verhandl. d. physiol. Gesellsch. in Berlin. His-Engelmann's Archiv, physiol. Abt., 1902, 544.

NEUBERG, C. *Die Physiologie der Pentosen und der Glukuronsäure.* Ergebnisse der Physiologie (Asher and Spiro), 1904, **3**, 1, 373.

NEUBERG, C., AND E. HIRSCHBERG. *Abbauversuche in der Kohlenhydratreihe.* Biochem. Zeitschr., 1910, **27**, 327.

NEUBERG, C., AND J. WOHLGEMUTH. *Ueber das Verhalten stereoisomerer Substanzen im Tierkörper. I. Ueber das Schicksal der drei Arabinosen im Kaninchenleibe.* Zeitschr. f. physiol. Chem., 1902, **35**, 41.

NEUBERG, C., AND P. MAYER. *Ueber das Verhalten stereoisomerer Substanzen im Tierkörper.* Zeitschr. f. physiol. Chem., 1903, **37**, 530.

NEUBERG, C. *Ueber die Umwandlung des Zuckers im Methylglyoxal.* Zeitschr. Ver. Dtsch. Zuckerind., 1915, 607.

NEUBERG, C. *Weitere Untersuchungen über die biochemische Umwandlung von Methylglyoxal in Milchsäure nebst Bemerkungen über die Entstehung der verschiedenen Milchsäuren in der Natur.* Biochem. Zeitschr., 1913, **51**, 484.

NICLOUX, M. *Injection de glycérine; dosage de la glycérine dans le sang; élimination par l'urine.* Compt. rendu Soc. Biol., 1903, **55**, 1014.

NICLOUX, M. *Injection intraveneuse de glycérine; dosage de la glycérine dans le sang; elimination par l'urine.* Compt. rendu, 1903, **136**, 559; **137**, 70.

NOORDEN, C. VON, AND G. EMBDEN. *Einige Probleme des intermediären Kohlehydratstoffwechsels.* Zentralbl. f. d. Physiol. u. Path. d. Stoffwechsels, New Series, 1906, **1**, 2.

OPPENHEIMER, M. *Ueber Milchsäurebildung in der künstlich durchströmten Leber.* Biochem. Zeitschr., 1912, **48**, 30.

OPPENHEIMER, M. *Ueber die Bildung von Glyzerin bei der alkoholischen Gärung.* Zeitschr. f. physiol. Chem., 1914, **89**, 63.

PADERI, C. *The behavior of gluconic acid in the organism.* Arch. Farmacol. sperim. 1915, **20**, 82.

PARNAS, J. *Ueber Bildung von Glykogen aus Glyzerinaldehyd in der Leber.* Zentralblatt f. Physiol., 1912, **26**, 671.

PARNAS, J., AND J. BAER. *Ueber Zuckerabbau und Zuckeraufbau im tierischen Organismus.* Biochem. Zeitschr., 1912, **41**, 386.

PINKUS, G. *Ueber die Einwirkung von Benzhydrazid auf Glucose.* Ber., 1898, **31**, 31.

POHL, J. *Ueber den oxydativen Abbau der Fettkörper im tierischen Organismus.* Arch. f. exper. Path. u. Pharm., 1896, **37**, 413.

RINGER, A. I., AND E. M. FRANKEL. *The formation of glucose from dioxyacetone in the diabetic organism.* Journ. Biol. Chem., 1914, **18**, 233.

ROSE, W. C. *Mucic acid and intermediary carbohydrate metabolism.* Journ. Biol. Chem., 1911, **10**, 123.

SALKOWSKI, E. *Ueber das Verhalten der Pentosen, insbesondere der l-Arabinose im Tierkörper.* Zeitschr. f. physiol. Chem., 1901, **32**, 393.

SALKOWSKI, E., AND NEUBERG. *Die Verwandlung von d-Glucuronsäure in l-Xylose.* Zeitschr. f. physiol. Chem., 1902, **36**, 261.

SANSUM, W. D., AND R. T. WOODYATT. *Glycollic aldehyde in phlorhizinised dogs.* Journ. Biol. Chem. 1914, **17**, 521.

SANSUM, W. D., AND R. T. WOODYATT. *The behaviour of glycerie aldhyde in the normal and diabetic organism.* Journ. Biol. Chem., 1916, **24**, 327, 343.

SCHMIEDEBERG, O., AND H. MEYER. *Ueber Stoffwechselprodukte nach Kampferfütterung.* Zeitschr. f. physiol. Chem., 1879, **3**, 422.

SCHMITZ, E. *Ueber den Mechanismus der Acrosebildung.* Ber., 1913, **46**, 2327.

SCHOTT, E. *Ueber das Verhalten der Glykonsäure und Zuckersäure im Organismus.* Arch. f. exper. Path. u. Pharm., 1911, **65**, 35.

SMEDLEY, I. *The action of the liver on the simpler sugars.* Journ. Physiol., 1912, **44**, 203.

TAYLOR, A. E. *On the derivation of ethyl alcohol contained in the muscle.* Journ. Biol. Chem., 1913, **15**, 217.

THIERFELDER, H. *Untersuchungen über die Glycuronsäure.* Zeitschr. f. physiol. Chem., 1887, **11**, 388.

WIEDEMANN. *Beiträge zur Pharmakologie des Kampfers.* Arch. f. exper. Path. u. Pharm., 1877, **6**, 216.

WINDAUS, A. *Ueber Saccharinbildung aus Hexosen.* Chem. Zeitschr., 1905, **29**, 564.

WIRTH, J. *Abbau von Kohlenhydratsäuren in der Leber.* Biochem. Zeitschr., 1911, **33**, 49.

REFERENCES TO CHAPTER V.

The Purine Derivatives.

Ackroyd, H. *Uric acid metabolism in dogs.* Biochem. Journ., 1910, **5**, 217.

Ackroyd, H. *Uric acid metabolism in rabbits.* Biochem. Journ., 1911, **5**, 422.

Ackroyd, H. *The presence of allantoin in certain foods.* Biochem. Journ., 1911, **5**, 400.

Ackroyd, H. *On the purine metabolism of rats.* Biochem. Journ., 1914, **8**, 434.

Ackroyd, H., and F. G. Hopkins. *Feeding experiments with deficiencies in the amino acids supply. Arginine and histidine as possible precursors of purines.* Biochem. Jour, 1916, **10**, 551.

Amberg, S., and W. Jones. *Ueber die bei der Spaltung der Nucleine in Betracht kommenden Fermente, mit besonderer Berücksichtigung der Bildung von Hypoxanthin in der Abwesenheit von Adenase.* Zeitschr. f. physiol. Chem., 1911, **73**, 407.

Ascoli, M., and G. Izar. *Quantitative Rückbildung zugesetzter Harnsäure in Leberextrakten und vorausgegangener Zerstörung.* Zeitschr. f. physiol. Chem., 1909, **58**, 529.

Ascoli, M., and G. Izar. *Beiträge zur Kenntnis der Harnsäurebildung.* Zeitschr. f. physiol. Chem., 1909, **62**, 347.

Austin, A. E. *The uricolytic enzyme in animal organs.* Journ. Med. Research., 1906, **15**, 309.

Austin, A. E. *The uricolytic enzyme.* II. Journ. Med. Research, 1907, **16**, 71.

Behrend, R., and R. Zieger. *Ueber die Oxydation der Harnsäure in alkalischer Lösung.* Liebig's Ann., 1915, **410**, 337.

Bezzol, C., G. Izar, and L. Preti. II. *Beiträge zur Kenntnis der Harnsäurebildung.* Zeitschr. f. physiol. Chem., 1909, **62**, 229.

Bloch, B. *Die Umwandlung der Purinkörper im Säugetierorganismus.* Sammelreferat. Biochem. Zentralbl., 1906, **5**, 521, 561, 817, 873.

Burian, R. *Ueber die oxydative und die vermeindlich synthetische Bildung von Harnsäure in Rinderleberauszug.* Zeitschr. f. physiol. Chem., 1904, **43**, 497.

Burian, R., and H. Schur. *Ueber die Stellung der Purinkörper im menschlichen Stoffwechsel.* II. *Die intermediäre Natur der Purinkörper des Säugetierstoffwechsels.* Pflüger's Archiv, 1901, **87**, 239.

Cohn, T. *Beitrag zur Kenntnis des Stoffwechsels nach Thymusnährung.* Zeitschr. f. physiol. Chem., 1898, **25**, 507.

Galeotti, G. *Versuche einer Isolierung des uricolytischen Fermentes.* Biochem. Zeitschr., 1911, **30**, 374.

Hirokawa, W. *Ueber den Einfluss langdauernder Nucleinsäurefütterung auf den Purinstoffwechsel und die Allantoinausscheidung beim Hunde.* Biochem. Zeitschr., 1910, **26**, 441.

Horbaczewski, J. *Untersuchungen über die Entstehung der Harnsäure im Säugetierorganismus.* Monatshefte f. Chemie, 1889, **10**, 624.

Horbaczewski, J. *Beiträge zur Kenntnis der Bildung der Harnsäure und der Xanthinbasen sowie der Entstehung der Leukocytose im Säugetierorganismus.* Monatshefte f. Chemie, 1891, **12**, 221.

Izar, G. *Beiträge zur Kenntnis der Harnsäurezerstörung und -bildung*. Zeitschr. f. physiol. Chem., 1911, **73**, 317.

Jones, W. *Concerning nucleases*. Journ. Biol. Chem., 1911, **9**, 129.

Jones, W. *On the physiological agents which are concerned in the nuclein fermentation, with special reference to four independent desamidases*. Journ. Biol. Chem., 1911, **9**, 169.

Jones, W., and C. L. Partridge. *Ueber die Guanase*. Zeitschr. f. physiol. Chem., 1904, **42**, 343.

Jones, W., and M. C. Winternitz. *Ueber die Adenase*. Zeitschr. f. physiol. Chem., 1905, **44**, 1.

Krüger, M. *Ueber den Abbau des Koffeins im Organismus des Hundes*. Ber., 1899, **32**, 2818.

Krüger, M., and J. Schmidt. *Ueber das Verhalten von Theobromin, Paraxanthin und 3-methylxanthin im Organismus*. Ber., 1899, **32**, 2677.

Krüger, M., and J. Schmidt. *Das Verhalten von Theobromin im Organismus des Menschen*. Arch. f. exper. Path. u. Pharm., 1901, **45**, 259.

Krüger, M., and J. Schmidt. *Die Entstehung der Harnsäure aus freien Purinbasen*. Zeitschr. f. physiol. Chem., 1902, **34**, 549.

Krüger, M., and J. Schmidt. *Der Abbau des Theophyllins, 1.3 dimethylxanthins, im Organismus des Hundes*. Ber., 1902, **36**, 1.

Levene, P. A., and J. A. Mandel. *Ueber die Konstitutton der Thymonucleinsäure*. Ber., 1908, **41**, 1905.

Levene, P. A., and F. Medegreceanu. *Nuclein metabolism in the dog*. Amer. Journ. Physiol., 1911, **27**, 438.

Lewis, H. B. *The behavior of some hydantoin derivatives in metabolism*. Journ. Biol. Chem., 1913, **13**, 347; **14**, 245, 1915; **23**, 281.

Luzatto, A. M. *Ueber das Verhalten des Allantoins im Tierkörper*. Zeitschr. f. physiol. Chem., 1903, **38**, 537.

Mendel, L. B., and J. F. Lyman. *The metabolism of some purine compounds in the rabbit, dog, pig and man*. Journ. Biol. Chem., 1910, **8**, 115.

Mendel, L. B., F. P. Underhill, and B. White. *A physiological study of nucleic acid*. Amer. Journ. Physiol., 1903, **8**, 376.

Mendel, L. B., and B. White. *On the intermediary metabolism of the purin bodies. The production of allantoin in the animal body*. Amer. Journ. Physiol., 1904, **12**, 85.

Michaelis, E. *Zur Frage des intermediären Purinstoffwechsels*. II. *Ueber den Harnsäurestich*. Zeitschr. f. exper. Path. u. Ther., 1913, **14**, 255.

Minkowski, O. *Untersuchungen zur Physiologie und Pathologie der Harnsäure bei Säugetieren*. Arch. f. exper. Path. u. Pharm., 1898, **41**, 375.

Nikolaier, A. *Ueber die Umwandlung des Adenins im tierischen Organismus*. Zeitschr. f. klin. Med., 1902, **45**, 359.

Plimmer, R. H. A., M. Dick, and C. C. Lieb. *A metabolism experiment with special reference to the origin of uric acid*. Journ Physiol., 1909, **39**, 98.

Poduschka, R. *Quantitative Versuche über Allantoinausscheidung*. Arch. f. exper. Path. u. Pharm., 1900, **44**, 59.

Preti, L. *Beiträge zur Kenntnis der Harnsäurebildung*. IV. Zeitschr. f. physiol. Chem. 1909, **62**, 354.

Salkowski, E. *Ueber das Verhalten in den Magen eingeführter Harnsäure im Organismus*. Zeitschr. f. physiol. Chem., 1902, **35**, 494.

Schittenhelm, A. *Ueber die Harnsäurebildung in Gewebsauszügen*. Zeitschr. f. physiol. Chem., 1904, **42**, 251.

Schittenhelm, A. *Ueber die Fermente des Nucleinstoffwechsels*. Zeitschr. f. physiol. Chem., 1904, **43**, 228.

Schittenhelm, A., and E. Bendix. *Ueber die Umwandlung des Guanins im Organismus des Kaninchens*. Zeitschr. f. physiol. Chem., 1904, **43**, 365.

SOETBEER, F., AND J. IBRAHIM. *Ueber das Schicksal eingeführter Harnsäure im menschlichen Organismus.* Zeitschr. f. physiol. Chem., 1902, **35**, 1.

SPITZER, W. *Die Ueberführung von Nucleinbasen in Harnsäure durch die Sauerstoff übertragende Wirkung von Gewebsauszügen.* Pflüger's Archiv, 1899, **76**, 192.

STEUDEL, H. *Das Verhalten einiger Pyrimidinderivate im Organismus.* Zeitschr. f. physiol. Chem., 1901, **32**, 285.

STEUDEL, H. *Fütterungsversuche in der Pyrimidingruppe.* Zeitschr. f. physiol. Chem., 1903, **39**, 136.

SUBKOW, L. *Ueber den Einfluss der Alkalien auf die Menge der ausgeschiedenen Harnsäure und über die Bedingungen der Zersetzungen der Harnsäure im Säugetierkörper.* Dissert. Moskow, 1903. Maly's Jahresber. f. Tierchemie, 1903, **33**, 873.

SUNDWIK, E. E. *Xanthinstoffe aus Harnsäure.* Zeitschr. f. physiol. Chem., 1912, **76**, 486.

THANNHAUSER, S. J., AND A. BOMMES. *Stoffwechselversuche mit Adenosin und Guanosin.* Zeitschr. f. physiol. Chem. 1914, **91**, 336.

WELLS, H. G. *The purine metabolism of the monkey.* Journ. Biol. Chem., 1910, **7**, 171.

WELLS, G. H., AND G. T. CALDWELL. *The purine enzymes of the orang-utan and chimpanzee.* Journ. Biol. Chem., 1914, **18**, 157.

WELLS, G. H. *The purine metabolism of the dalmatian coach hound.* Journ. Biol. Chem., 1918, **35**, 221.

WIECHOWSKI, W. *Die Produkte der fermentativen Harnsäurezersetzung durch tierische Organe.* Beiträge z. chem. Physiol. u. Path., 1907, **9**, 295.

WIECHOWSKI, W. *Die Bedeutung des Allantoins im Harnsäurestoffwechsel.* Beiträge z. chem. Physiol. u. Path.. 1907, **11**, 109.

WIECHOWSKI, W. *Ueber die Zersetzlichkeit der Harnsäure im menschlichen Organismus.* Arch. f. exper. Path. u. Pharm., 1909, **60**, 185.

WIECHOWSKI, W. *Das Schicksal intermediärer Harnsäure beim Menschen und der Allantoingehalt des menschlichen Harnes; nebst Bemerkungen über Nachweis und Zersetzlichkeit des Allantoins.* Biochem. Zeitschr., 1910, **25**, 431.

WIECHOWSKI, W., AND H. WIENER. *Ueber Eigenschaften und Darstellung des Harnsäure zerstörenden Fermentes der Rinderniere und Hundeleber.* Beiträge z. chem. Physiol. u. Path., 1907, **9**, 247.

WIENER, H. *Ueber Zersetzung und Bildung der Harnsäure im Tierkörper.* Arch. f. exper. Path. u. Pharm., 1899, **42**, 375.

WIENER, H. *Die Harnsäure.* Ergebnisse der Physiologie, 1902, **1**, 555.

WIENER, H. *Die Harnsäure in ihrer Bedeutung für die Pathologie.* Ergebnisse der Physiologie, 1903, **2**, 377.

REFERENCES TO CHAPTER VI.

HYDROCARBONS, PHENOLS, ALCOHOLS, ALDEHYDES, AMINES AND INDOLE DERIVATIVES.

ABELOUS, J. E., AND J. ALOY. *Sur quelques conditions de l'activité d'un ferment oxydant.* Compt. rend. soc. biol., 1903, **55**, 891.

ABELOUS, J. E., AND G. BIARNÈS. *Sur l'existence ches les mammifères d'une oxydaseglobuline, ses caractères et ses propriétés.* Arch. de physiol., 1898, **30**, 664.

BARGER, G., AND H. D. DAKIN. *Note on some experiments with glyoxaline derivatives.* Biochem. Journ., 1916, **10**, 376.

BATTELLI, F., AND F. STERN. *Die Alkoholoxydase in den Tiergeweben.* Biochem. Zeitschr., 1910, **28**, 145.

BATTELLI, F., AND L. STERN. *Die Aldehydase in den Tiergeweben.* Biochem. Zeitschr., 1910, **29**, 130.

BATTELL, F., AND L. STERN. *Dédoublement de l'aldehyde éthylique en acide et alcool par les tissues animaux.* Soc. de Biol., 1910, **68**, 742.

BAUMANN, E. *Ueber die Synthese der Aetherschwefelsäure der Phenole.* Ber., 1876, **9**, 1715.

BAUMANN, E., AND E. HERTER. *Ueber die Synthese von Aetherschwefelsäuren und das Verhalten einiger aromatischer Substanzen.* Zeitschr. f. physiol. Chem., 1878, **1**, 244.

BAUMANN, E., AND C. PREUSSE. *Ueber die dunkle Farbe des "Karbolharns".* Du Bois Raymond's Archiv f. Physiol., 1879, 245.

BAUMANN, E., AND L. BRIEGER. *Ueber Indoxylschwefelsäure, das Indican des Harns.* Zeitschr. f. physiol. Chem., 1879, **3**, 254.

BLUMENTHAL, F., AND E. JACOBY. *Beiträge zum Nachweis und zur Entstehung aromatischer Körper im Organismus.* II. Mitteilung. *Verhalten von Indol und Skatol beim Kaninchen.* Biochem. Zeitschr., 1910, **29**, 472.

BRIEGER, L. *Weitere Beiträge zur Kenntnis des Skatols.* Zeitschr. f. physiol. Chem., 1880, **4**, 414.

BUCHNER, E., AND R. GAUNT. *Ueber die Essiggärung.* Annalen, 1906, **349**, 140.

CROSS, C. F., E. J. BEVAN, AND T. HEIBERG. *Einwirkung von Wasserstoffhydroperoxyd auf ungesättigte Kohlenwasserstoffe.* Ber., 1900, **33**, 2015.

DONY-HENAULT, O., AND J. VAN DUUREN. *Contribution à l'étude methodique des oxydases dans les tissus animaux.* Bull. de l'Acad. royale de Belgique, 1907, 537. Arch. int. de Physiol., 1907, **5**, 39.

EMBDEN, G., AND K. BALDES. *Ueber Umwandlung von Acetaldehyd in Aethylalkohol im tierischen Organismus.* Biochem. Zeitschr., 1912, **45**, 157.

ERDMANN, C. C. *On the determination of alkylamines obtained from urine after Kjeldahl digestion.* Journ. Biol. Chem., 1911, **9**, 85.

EWINS, A. J., AND P. P. LAIDLAW. *The fate of parahydroxyphenylethylamine in the organism.* Journ. of Physiol., 1910, **41**, 78.

EWINS, A. J., AND P. P. LAIDLAW. *The fate of indolethylamine in the organism.* Biochem. Journ. 1913, **7**, 18.

FRIEDEL, C., AND J. M. CRAFTS. *New general method for the synthesis of aromatic compounds.* Annales de Chemie et de Physique, 1888 (6), **14**, 435

FRIEDMANN, K., AND W. TURK. *Verhalten des Benzaldehyds im Tierkörper*. Biochem. Zeitschr., 1913, **55**, 425.

GATTERMANN, L., AND F. FRIEDRICHS. *Ueber die Einwirkung des elektrischen Stromes auf Benzol*. Ber., 1894, **27**, 1942.

GERGENS, E., AND E. BAUMANN. *Ueber das Verhalten des Guanidin, Dicyandiamidin und Cyanamid im Organismus*. Pflüger's Archiv, 1876, **12**, 205.

GUGGENHEIM, M., AND W. LOEFFLER. *Das Schicksal proteinogener Amine im Tierkörper*. Biochem. Zeitschr. 1915, **72**, 325.

HEIMROD, G. W., AND P. A. LEVENE. *Ueber die Oxydation von Aldehyden in alkalischer Lösung*. Biochem. Zeitschr., 1910, **29**, 31.

HERTER, C. A. *On indolacetic acid as the chromogen of the "Urorosein" of the urine*. Journ. of Biol. Chem., 1908, **4**, 253.

HOPPE-SEYLER, F. *Ueber Gährungsprozesse*. Zeitschr. f. physiol. Chem., 1878, **2**, 1.

JACOBY, M. *Ueber das Aldehyde oxydierende Ferment der Leber und Nebenniere*. Zeitschr. f. physiol. Chem., 1900, **30**, 135.

JAFFÉ, M. *Ueber die Aufspaltung des Benzolringes im Organismus. Das Auftreten von Muconsäure im Harn nach Darreichung von Benzol*. Zeitschr. f. physiol. Chem., 1909, **62**, 58.

JAFFÉ, M. *Ueber den Nachweis und die quantitative Bestimmung des Indicans im Harn*. Pflüger's Archiv, 1870, **3**, 448.

JAFFÉ, M. *Ueber das regelmässige Vorkommen von Indol im Destillate des normalen Harns*. Arch. f. exper. Path. u. Pharm., 1908, Supplementband, Schmiedeberg's Festschrift, 299.

JAQUET, A. *Ueber die Bedingungen der Oxydationsvorgänge in den Geweben*. Arch. f. exper. Path. u. Pharm., 1891, **29**, 386.

KRIMBERG, R. *Zur Kenntnis der Extraktivstoffe der Muskeln*. IV. Mitteilung. *Ueber das Vorkommen des Carnosins, Carnitins und Methylguanidins im Fleisch*. Zeitschr. f. physiol. Chem., 1906, **48**, 412.

KULZ, E. *Ueber einige gepaarte Glycuronsäuren*. Zeitschr. f. Biol., 1890, **27**, 247.

LASSAR, O. *Ueber den Zusammenhang von Hautresorption und Albuminurie*. Virchow's Archiv, 1879, **77**, 157.

LEEDS, A. R. *Ueber die Einwirkung von Ozon, naszierenden Sauerstoff und Wasserstoffhydroperoxyden auf Benzol*. Ber., 1881, **14**, 975.

LEWIN, L. *Ueber allgemeine und Hautvergiftung durch Petroleum*. Virchow's Archiv, 1888, **112**, 35.

LIFSCHÜTZ, J. *Die Oxydationsprodukte des Cholesterins in den tierischen Organen*. Biochem. Zeitschr., 1913, **52**, 206.

LIFSCHÜTZ, J. *Die Oxydation des Cholesterins durch das Blutgewebe*. Zeitschr. f. physiol. Chem., 1915, **93**, 209.

MASUDA, N. *Ueber das Auftreten aldehydartiger Substanzen bei der Leberdurchblutung und über Acetessigsäurebildung aus Aethylalkohol*. Biochem. Zeitschr., 1912, **45**, 140.

MEDWEDEW, A. *Ueber die oxydativen Leistungen im tierischen Gewebe*. II. Mitteilung. Pflügers's Archiv, 1900, **81**, 540.

MORI, Y. *The decomposition of muconic and adipic acids in the animal body*. Journ. Biolog. Chem., 1918, **35**, 341.

MOSSO, U. *Quantitative Untersuchungen über die Ausscheidung der Salizylsäure und der Umwandlungsprodukte des Benzylamins aus dem tierischen Organismus*. Arch. f. exper. Path. u. Pharm., 1889, **26**, 267.

MUNK, J. *Zur Kenntnis der phenolbildenden Substanz im Harn*. Pflüger's Archiv 1896, **12**, 142.

NENCKI, M., AND P. GIACOSA. *Ueber die Oxydation der aromatischen Kohlenwasserstoffe im Tierkörper*. Zeitschr. f. physiol. Chem., 1880, **4**, 326.

NENCKI, M., AND P. GIACOSA. *Ueber die Oxydation des Benzols durch Ozon und die Oxydationen im Tierkörper*. Zeitschr. f. physiol. Chem., 1880, **4**, 339.

NENCKI, M., AND N. SIEBER. *Ueber eine neue Methode, die physiologische Oxydation zu messen, und über den Einfluss der Gifte und Krankheiten auf dieselbe* Pflüger's Archiv, 1883, **31**, 319.

NENCKI, M., AND N. SIEBER. *Untersuchungen über die physiologische Oxydation.* Journ. f. prakt. Chem., 1882, **26**, 1.

NEUBAUER, O. *Ueber Glykuronsäurepaarung bei Stoffen der Fettreihe.* Arch. f. exper. Path. u. Pharm., 1901, **46**, 133.

NEUBERG, C., AND E. SCHWENK. *Ueber Indoxylglukuronsäure.* Biochem. Zeitschr. 1916, **79**, 383.

PARNAS, J. *Ueber fermentative Beschleunigung der Cannizaroschen Aldehydumlagerung durch Gewebssäfte.* Biochem. Zeitschr., 1910, **28**, 274.

POHL, J. *Ueber die Oxydation des Methyl- und Aethylalkohols im Tierkörper.* Arch f. exper. Path. u. Pharm., 1893, **31**, 281.

POHL, J. *Zur Kenntnis des oxydativen Ferments.* Arch. f. exper. Path. u. Pharm., 1896, **38**, 65.

POMMERENIG, E. *Ueber Guanidinzersetzung im Tierkörper.* Beitr. z. chem. Physiol. u. Path., 1901, **1**, 561.

PORCHER, C. *Recherches sur l'indol.* Bull. Soc. Chim., 1909, IV, **5**, 526.

SACHS, F. *Ueber den Chemismus des Leucinabbaues in Leber.* Biochem. Zeitschr., 1910, **27**, 27.

SALKOWSKI, E. *Ueber den Vorgang der Harnstoffbildung und den Einfluss der Ammoniaksalze auf denselben.* Zeitschr. f. physiol. Chem., 1877, **1**, 1.

SALKOWSKI, E., AND C. NEUBERG. *Zur Kenntnis der Phenolglucuronsäure.* Biochem. Zeitschr., 1906, **2**, 307.

SCHAFFER, FR. *Ueber die Ausscheidung des dem Tierkörper zugeführten Phenols.* Journ. f. prakt. Chem., 1878, **18**, 282.

SCHIFFER, J. *Ueber das Vorkommen und die Entstehung von Methylamin und Methylharnstoff im Harn.* Zeitschr. f. physiol. Chem., 1880, **4**, 237.

SCHMIEDEBERG, O. *Das Verhältnis des Ammoniaks und der primären Monoaminbasen zur Harnstoffbildung im Tierkörper.* Arch. f. exper. Path. u. Pharm., 1877 **8**, 1.

SCHMIEDEBERG, O. *Ueber Oxydationen und Synthesen im Tierkörper.* Arch. f. exper. Path. u. Pharm., 1881, **14**, 288.

SCHMIEDEBERG, O. *Ueber Oxydationen und Synthesen im Tierkörper.* Arch. f. exper Path. u. Pharm., 1881, **14**, 306.

SCHULTZEN, O., AND B. NAUNYN. *Ueber das Verhalten der Kohlenwasserstoffe im tierischen Organismus.* Reichert's and Du Bois Raymond's Archiv, 1867, 349.

SOBIERANSKI, W. *Ueber die Resorption des Vaselins von der Haut und seine Schicksale im Organismus.* Arch. f. exper. Path. u. Pharm., 1893, **31**, 329.

SPITZER, W. *Die Bedeutung gewisser Nucleoproteide für die oxydative Leistung der Zelle.* Pflüger's Archiv, 1897, **67**, 615.

SUTO, K. *Ueber die Oxydation von Aminen.* Biochem. Zeitschr., 1915, **71**, 169.

TAUBER, E. *Beiträge zur Kenntnis über das Verhalten des Phenols im tierischen Organismus.* Zeitschr. f. physiol. Chem., 1878, **2**, 366.

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